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FEVER, INFECTIONS AND THE THYROID-ADRENAL APPARATUS.

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IN previous papers we have developed the conception that the thyroid and adrenal glands are endocrine factors in the heat regulation of the body (Cramer, 1916–1924). This conception may be briefly outlined as follows: All the factors affecting the heat regulation of the body—both the chemical heat regulation and the physical heat regulation—are under the control of the sympathetic. They are, therefore, affected by the activity of the thyroid and adrenal glands, which have a synergetic action. By their increased functional activity heat production is increased and heat loss diminished. Thus the injection of a substance such as β-tetrahydronaphthylamin, which stimulates these glands to active secretion, produces a fever which from its origin we have called "sympathetic fever." When, on the other hand, the organism is exposed to conditions such as cold, which call for increased heat production and diminished heat loss, the thyroid and adrenal gland are stimulated to active secretion. This increased functional activity can be demonstrated in the adrenal medulla, which shows a massive secretion of adrenalin into the central vein and its tributaries. There is also congestion of the capillaries in the zona reticularis, an opening up of the capillary blood-spaces in the medulla, which in the resting gland are closed, and a migration of the cortical lipoid from the periphery towards the medulla, resulting sometimes in the appearance of large globules of lipoid in the zona reticularis. In the thyroid gland, when active secretion is induced by exposure to cold or by the injection of β-tetrahydronaphthylamin, the colloid at first loses its staining affinity for Heidenhain's hæmatoxylin and later disappears from the alveoli. There is also dilatation of the intra-alveolar capillaries so that they lift up the epithelium lining the alveoli from the basement membrane. This sometimes leads to desquamation of the epithelial cells and small hæmorrhages into the alveolar lumen. There is also congestion of the inter-alveolar capillaries. These various changes are illustrated in Figs. 1–5.

In one of our earlier papers (Cramer, 1919) we produced evidence that the disturbances of the normal heat regulation in many bacterial infections are due to the action of bacterial toxins on the thyroid adrenal apparatus. Since then we have collected further experimental and pathological evidence bearing on this point. Our conception of the control of body temperature by the adrenal gland as an endocrine factor has been adopted recently *in toto*, but without acknowledgment, by Cannon (1924). In the present paper we propose to deal more fully with the application of this conception to pathological conditions involving disturbances in the heat-regulating mechanism. We may conveniently deal with this pathological aspect under two headings: firstly, those conditions which are primarily diseases of the thyroid and adrenal glands, and secondly, those conditions which are primarily disturbances of the heat-regulating mechanism. The latter are represented mainly by the fevers due to bacterial infections.

HEAT REGULATION IN GRAVES'S DISEASE, MYXŒDEMA AND ADDISON'S DISEASE.

It is well known that the heat-regulating mechanism is affected in myxœdema, in Addison's disease, and in Graves's disease. In Graves's disease the increased metabolism of hyperthyroidism and the increased muscular activity (tachycardia, muscular tremor) mean increased heat production; in other words, the chemical heat regulation is stimulated to increased activity. The physical heat regulation is also stimulated, for the cutaneous blood-vessels are dilated, there is increased perspiration, and respiration is increased. The increased loss of heat resulting from these various disturbances does not always compensate for the increased heat production, so that the temperature may rise above the normal, resulting in periods of actual fever. In fact, clinically, the symptoms of hyperthyroidism may simulate those of early tuberculosis so nearly that attempts have been made to devise special tests in order to distinguish between these two conditions (Goetsch, 1920). We shall see later in dealing with the condition of the thyroid in bacterial infections that this similarity between these two apparently so dissimilar conditions is not an accidental one.

In myxœdema all these conditions are reversed. The chemical heat regulation is depressed as the result of the diminished basal metabolism and the sluggish muscular activity. Instead of the flushed, warm, moist skin of the hyperthyroid patient which promotes heat loss we have the cold, thickened, dry skin with diminished heat loss. The temperature is subnormal.

The deficient functioning of the adrenals in Addison's disease is also accompanied by a subnormal temperature, which is associated with and may partly be the result of muscular asthenia.

ADRENAL HÆMORRHAGE AND HEAT REGULATION.

Hyperadrenalism as a primary disease is not recognized as a clinical entity in the same way as hyperthyroidism. This may be due to the fact that the morbid histology of the adrenal presents a much more difficult problem than the thyroid gland. There are, however, on record conditions in which the

only lesion found post-mortem is intense congestion of the adrenals with hæmorrhages into the glands. An outstanding symptom which is frequently associated with this condition is a sudden hyperpyrexia, which may occur in the absence of any demonstrable infection, or which is too violent to be accounted for by any infection that may be present. The patient may die in a day or two, the general clinical picture being that of a virulent infection, for which it is frequently mistaken. The hæmorrhage may be visible as such to the naked eye, or it may only be seen on microscopic examination—the naked-eye appearance only showing intense congestion. My limited pathological material includes two such cases. One was an infant which was admitted to the hospital with a temperature of 104°, which had developed rapidly a few hours after admission. No evidence of any infection could be found post mortem. The other case was that of a man, aged 60, who had been operated on for enlarged prostate. The operation was successful, and the temperature remained normal for the succeeding seven days. Then the temperature suddenly rose, and the patient died on the following day. It was suspected that an infection had supervened, but no evidence of this could be found at the post-mortem examination. In both cases the zona reticularis and the medulla of the adrenals showed intense congestion with diffuse hæmorrhage into the medulla.

This condition of diffuse adrenal hæmorrhage associated with congestion is perhaps more frequent than the relatively few number of cases recorded in the literature indicate. It is rarely, if ever, diagnosed during life. The adrenal is not always examined carefully at autopsies, and unless the hæmorrhage is visible to the naked eye it would be missed. Sometimes the fever may subside and be followed by a subnormal temperature and asthenia, so that the patient dies with the symptoms of adrenal insufficiency. Such a case in which post-mortem a large macroscopic hæmorrhage into the adrenals was found has been described by Hektoen (1910). The patient had been operated on for an inguinal hernia. The temperature remained normal for three days after the operation, then rose to 102°; it then became normal until shortly before death fourteen days after the operation, when it fell below normal. The history of this case suggests that the initial stimulation of the gland was followed by a slow hæmorrhage into it which gradually destroyed the medulla. Severn (1923) records an instructive case of acute bilateral suprarenal hæmorrhage which simulated an acute infection in its symptoms: a pyrexia of 102°, a rapid pulse of 126, and cold face and extremities, followed by collapse. Another group of cases is distinguished by the occurrence of purpuric eruptions (Graham Little, 1901), either with or without a rise in temperature. In the former group of cases death ensues frequently in a few days. Such cases have often been recorded as purpuric smallpox or hæmorrhagic scarlatina. Dudgeon (1904), however, has pointed out that in many of these cases there has been no clear evidence that the patients had either smallpox or scarlatina, and he suggests that they may have been cases of adrenal hæmorrhage pure and simple unaccompanied by any infections.

Most cases of adrenal hæmorrhage in adults recorded in the literature refer to large hæmorrhages visible to the naked eye, to which the name "adrenal apoplexy" has been applied by some authors. A systematic microscopic

investigation of the adrenal would probably show a considerable number of cases of diffuse hæmorrhages which have so far escaped detection. From a study of the cases recorded in the literature it is clear that adrenal hæmorrhage may be the result of a variety of causes. Thrombosis of the adrenal vein is a frequent cause. Disease of the blood-vessels due, for instance to syphilis is another predisposing cause. An instructive case of this kind may be given in which a man, aged 40, with a history of syphilis and suffering from pyorrhœa, developed a temperature of 102·5° and a pulse of 120. Post-mortem examination showed hæmorrhage into the adrenal with thrombosis of the adrenal vein. Destructive disease of the gland may also conceivably lead to a hæmorrhage into the gland. Another cause is the action of a toxin on the endothelial lining of the capillary spaces in the gland, especially when it is associated with the dilatation of these spaces, and the congestion of the gland which, as will be shown presently, accompany many of the bacterial infections. One of the earliest cases recorded in the literature is that of a man, aged 29, who died of pneumonia (Greenhow, 1877). Post-mortem examination revealed a hæmorrhage into the suprarenals. Of the numerous cases collected by Arnaud, two were observed as having occurred after extensive burns, while one was found in a person frozen to death ("coup de froid"). The paper by Arnaud (1900) gives a very complete collection of all cases recorded in the literature up to 1900. Since then a number of other cases have been reported, in addition to those mentioned above (Pritchard, 1890; Simmonds, 1902; Dudgeon, 1904; Lissauer, 1908; Lavenson, 1900; Brodnitz, 1910; Goldzieher, 1911; Freudemann, 1920; Michaux and Marsset, 1923).

Of particular interest is the occurrence of hæmorrhages into the adrenals which has been observed in malaria (Paisseau and Lemaire, 1916; Dudgeon and Clarke, 1917) and in influenza (Winter, 1918; Wolbach, 1919)—two conditions which are particularly associated with an intense hyperthermia of the type which, from our experimental observations, one would expect to be associated with great activity of the suprarenals. Generally speaking, therefore, adrenal hæmorrhage is particularly apt to occur in certain bacterial infections which involve intense stimulation of the gland. In these cases the pyrexia could be accounted for as a symptom of the bacterial infection. But, as has been stated above, there are cases of adrenal hæmorrhage which have been preceded by a pyrexia developing suddenly in the absence of a demonstrable infection. In such cases the pyrexia may in the light of our experimental observation be attributed to a sudden hyperæmia of the adrenals due to some intrinsic cause; it is an "aseptic fever." This accounts, as we shall see presently, for the frequent occurrence of adrenal hæmorrhage in the newborn. The abnormal production of a substance, which, like β-tetrahydronaphthylamin, has a strong stimulating action of the adrenals, must be considered as a possibility in some cases. We know from the work of Barger and Dale (1910), on sympathomimetic amines, that intestinal putrefaction may produce from the amino-acids of proteins amines having a powerful action on the sympathetic system.

It is curious that of the relatively few cases of adrenal hæmorrhage recorded in the literature, a great many refer to babies soon after birth (Still, 1897; Garrod and Drysdale, 1897; Arnaud, 1900; Hamill, 1901; Dudgeon, 1904; Litzenberg and White, 1908; Friederichsen, 1918; Rabinowitz, 1923). This

prevalence apparently has led to the belief that this condition is due to mechanical injuries received at birth. It is difficult to see why the adrenal of all organs should be liable to such injuries at birth. The importance of the adrenal gland as an endocrine factor in heat regulation offers a more satisfactory explanation. As long as the fœtus is *in utero* no call is made on the heat-regulating mechanism. But such a demand is suddenly made at birth, and during the first few weeks after birth an extraordinary process of involution of that part of the fœtal adrenal which lies between the medulla and the permanent cortex, transforms the adrenal of the human fœtus into that of the typical gland. This process is accompanied by an intense congestion, particularly at the junction of medulla and cortex, for which the sudden change in the environment at and after birth is probably the stimulus. It is easy to see how this normal process may be carried too far and lead to a hæmorrhage into the adrenals. The clinical observations of both Friederichsen (1918) and of Corcoran and Strauss (1924) show also that one of the symptoms of adrenal hæmorrhage is fever. In the case of a newborn baby, which the latter authors report, the temperature rose to 105° F. on the fourth day after birth. The hæmatoma was removed by operation, which was followed by complete recovery. In this case there was no evidence of an infection, but in some of the cases reported in the literature the hæmorrhage must be attributed to a concurrent infection.

These clinical and pathological observations may be summarized as showing that adrenal hæmorrhages are almost always accompanied by a sudden fever, in which the patient may die. It may even be accompanied by purpuric eruptions. Sometimes the fever subsides, and is followed by asthenia or collapse with a subnormal temperature. Adrenal hæmorrhage is a lesion which may occur in the course of bacterial infections; it has also been found in the absence of such infections. In the latter case the fever may be described as an "aseptic fever." What, then, is the relation of such an "aseptic fever" to the "septic fevers" of bacterial infections? Is the underlying process the same, and related in both conditions to an increased activity of the adrenal leading to congestion and hæmorrhage? Or are the two processes fundamentally different, and is the similarity of the adrenal lesion merely an accident which has no significance?

SEPTIC AND ASEPTIC FEVERS: SIMILARITY OF METABOLIC CHANGES IN MALARIAL FEVER AND IN SYMPATHETIC FEVER.

It has been held by some writers that the processes underlying the fevers due to bacterial infections are fundamentally different from the aseptic fevers, but are essentially similar in the various bacterial infections. On this assumption the various bacterial toxins are supposed to act in an essentially similar manner on the heat-regulating mechanism. This *à priori* conception of an essential similarity of the mechanism underlying the different "septic" fevers and of a fundamental distinction between them and the aseptic fevers seems to us ill-founded. For the several types of fevers produced by various bacterial toxins differ as widely from each other as they differ from the aseptic fevers. The hectic type of fever in tuberculosis and the rigor of malaria or of an acute streptococcal infection represent two very different types of disturbance

of the heat-regulating mechanism. The same difference in type exists in aseptic fevers produced in different ways: the hyperpyrexia elicited by the injection of β-tetrahydronaphthylamin corresponds to the malarial rigor, while prolonged thyroid feeding produces an entirely different type of fever (Cramer, 1924), resembling the hectic type of tubercular infections. There are, moreover, bacterial infections such as diphtheria or gas gangrene in which, after an initial fever, the course of the infection may be accompanied by a subnormal temperature. Again, observations on the metabolism in fever have failed to produce any convincing evidence that the chemical heat regulation in bacterial infection is fundamentally different from that of the normal organism.

The complete calorimetric investigation of fevers in man presents obvious technical difficulties, and most investigations deal only with isolated aspects of the metabolism of fever. Great credit is due to the workers in the Russel Sage Institute of Pathology in New York for having overcome these difficulties by the construction of a respiration calorimeter capable of measuring the rapid and irregular metabolic changes occurring in fever. Among other fevers they have investigated the metabolism in malaria (Barr and Dubois, 1918). This is of special interest, as the different phases of a malarial attack exhibit many different types of fever. Their results are briefly as follows: During the chill, when the rectal temperature rises abruptly, the heat production is rapidly increased 100 to 200 per cent. In spite of this rapid increase the heat elimination, however, is not increased, the surface temperature is not increased but may actually show a fall. After the chill the surface becomes warmer, the heat elimination increases slowly until it equals the heat production, so that the temperature remains high. With the onset of sweating the heat loss is enormously increased and exceeds heat production so that the temperature falls. After the attack both heat production and heat loss return to the normal. The respiratory quotient is higher during the chill than before or immediately after it, indicating an increased oxidation of carbohydrates. The nitrogen excretion was increased during the attack in spite of an ample intake of food, and in two cases this increase persisted for several days after the attack. A constriction of the peripheral blood-vessels during the chill was demonstrated long ago in 1888 by Maragliano by means of the plethysmograph. It is interesting to note that this constriction begins actually before the chill, and that similarly the rise in the respiratory quotient sets in before the chill.

It will be seen that all the metabolic features of malarial fever during the chill are a replica of those found in experimental "sympathetic fever" after the injection of β-tetrahydronaphthylamin. Even the persistent increase in the nitrogen excretion occurs in the latter condition. It is, as we have seen (Cramer, 1924), the result of the increased activity of the glycogenic function, and need not be attributed to a *toxic* protein destruction. These very complete observations indicate that the fever of a malarial paroxysm involves the same mechanism which is set in action when an animal is exposed to cold or when sympathetic fever is produced experimentally. It is not suggested here that all bacterial toxins produce fever in the same way. Some may do so by a peripheral action on the blood-vessels, on the sweat-glands, or by inducing abnormal metabolic processes. We may gain further evidence on

this question whether the thyroid and adrenal glands are involved in the various types of bacterial infections by studying the appearance of these glands in different infections.

ADRENAL GLAND IN BACTERIAL INFECTIONS.

The most convincing evidence is obtained from experimental infections, since in human material obtained post mortem the actual cause of death may be some accessory or accidental factor which has arisen in the course of the infection, and which may itself produce an effect on the thyroid or adrenal glands, and thus obscure the changes due to the bacterial infection itself. For this reason the changes produced by acute infections which have led rapidly to a fatal issue are of special value when dealing with human material.

There are two bacterial toxins which can be shown experimentally to produce an exhaustion of the suprarenal gland—diphtheria toxin, and the toxin of *B. Welchii* of gas gangrene. The similarity in this " adrenalotoxic " action is reflected in the similarity of the temperature reaction in the course of these two infections. After a brief initial fever the temperature falls and becomes subnormal, especially if the issue is fatal. It is a remarkable fact that mice, while very susceptible to the toxin of *B. Welchii*, are absolutely resistant to diphtheria toxin. A dose of diphtheria toxin which would kill one hundred guinea-pigs can be injected into a mouse without producing any obvious effects on the animal, and, what is equally remarkable, without producing any change in the suprarenals. This fact suggests that the reaction of the adrenal gland to diphtheria toxin is an important factor in determining the effect of the toxin on the animal as a whole. In other words, the pathogenic effect of the diphtheria bacillus is dependent, in part at any rate, on the susceptibility of the adrenal gland to its toxin. This view is confirmed by observations made with the toxin of *B. Welchii*. It could be shown experimentally (Bullock (Gye) and Cramer, 1919) that a sublethal dose of this toxin could have a lethal effect in mice if the animals were subjected at the same time to conditions such as exposure to cold, which imposed an additional strain upon the adrenals.

The toxin of *B. Welchii* affects not only the medulla, where the adrenalin disappears from the cells, but also the cortex, from which the lipoid disappears almost completely. In addition there is congestion of the gland. Diphtheria toxin produces in the guinea-pig adrenal congestion, with small hæmorrhages in the medulla. The cortex shows a disappearance of the lipoid.

Another type of infection has been studied by us experimentally, namely streptococcal infections of varying degree of virulence. Both the strains used when injected by themselves produced a localized abscess and a rise of temperature, without, however, killing the animals. The adrenals showed an active secretion of adrenalin, but with the more virulent strain the medulla secreted more actively so that it was partially depleted. In both conditions the cortical lipoid had not disappeared.

The conditions were varied further in such a way as to induce a streptococcal cellulitis by injecting an ionizable calcium salt together with the bacteria, and thus producing the phenomenon " kataphylaxis " or " defence-rupture."

This eventually led to a streptococcal septicæmia, and with the more virulent strain killed the animals within four days in a condition of collapse. In these animals the medulla was found to be completely exhausted, and the lipoid had disappeared from the cortex. The gland in fact resembled in appearance those of animals which had died from the effects of gas gangrene toxin. The septicæmia resulting from the less virulent strain did not kill the animals within four days, although they were obviously ill, and in these mice the adrenals were not exhausted; the medulla showed active secretion and the cortical lipoid was not obviously diminished.

The literature contains several statements (Goldzieher, 1911; Weltmann, 1913; Elliot, 1914), describing similar profound changes in the adrenals in experimental infections with other bacteria (anthrax, pneumococcus, *B. pestis*, staphylococci, streptococci, Shiga bacillus, typhoid). The results of the various authors may be summarized by saying that experimental infections so virulent or so massive that they lead to death in a few days produce, if the infection is allowed to run its course, a disappearance of the cortical lipoids together with a depletion of adrenalin from the medulla. But if intermediate stages of these infections are examined, the cortical lipoid is found to be increased and to have spread over the cortex. A particularly intense hyperæmia with hæmorrhages had been observed in experimental infections with the pneumococcus. It is of interest to note that even the toxins of intestinal worms (*Ascaris*, *Tænia*) have been found to produce a disappearance of the cortical lipoid (Bedson, 1913).

The conclusions which one may draw from these observations are that the adrenal gland is particularly susceptible to the action of many bacterial toxins. Small doses of many bacterial toxins stimulate the gland to increased activity, while in lethal doses they exhaust the gland. One might describe these effects as "adrenalotonic" and "adrenalotoxic" respectively. There are toxins in which the adrenalotoxic effect predominates. But even a toxin giving an adrenalotonic effect, if its action is sufficiently powerful and prolonged, may eventually lead to an exhaustion of the gland.

For those infections that have an action on the adrenals, there is therefore some evidence that what is vaguely described as their "virulence" is determined, in part at any rate, by their action on the adrenals, and that the exhaustion of the adrenals is an important factor in the lethal issue, which is usually ascribed vaguely to a failure of the circulation. It is hardly necessary to add that there are bacterial toxins, of which tetanus toxin is an example, which produce their toxic action in quite a different manner, or that those infections that have an adrenalotoxic effect may also have toxic effects on other organs.

The observations also give important information on what constitutes exhaustion of the adrenal gland. It is not merely depletion of the load of adrenalin from the medulla. For this may be brought about by an increased secretory activity of the gland, as, for instance, by exposure to cold or by a dose of β-tetrahydronaphthylamin. If, for instance, after a severe exposure to cold the animal is kept warm it recovers its normal body temperature, and the adrenal, medulla fills itself again with adrenalin. And after a dose of β-tetrahydronaphthylamin sufficient to deplete profoundly the load of adrenalin, the body temperature does not fall below the normal after it has recovered from the period of pyrexia and the medulla assumes its normal load

of adrenalin. In the bacterial infections which have been studied experimentally by us, those that lead to the death of the animal with a greatly subnormal temperature and in a state of collapse show a depletion of adrenalin from the medulla which is always accompanied by a disappearance of lipoid from the cortex. As shown in the preceding paper (Cramer, 1926), the presence of lipoid in the cortex is essential for the proper functioning of the medulla. In its absence the rapid new formation of adrenalin which normally accompanies the active secretion of adrenalin appears to be impaired, so that a condition of adrenal exhaustion or adrenal insufficiency is produced. Its visible manifestation is the absence of lipoid from the cortex and of adrenalin from the medulla. Such a condition of the gland seems to be incompatible with life.

If one examines intermediate stages in the action of these adrenalotoxic toxins, one finds the lipoids spreading first from the outer cells of the zona fasciculata of the cortex over the whole cortex, and appearing in the inner cells of this zone and in the cells of the zona reticularis, which are normally free from it. There is thus a movement of the lipoid from the periphery towards the medulla, but not as a rule into the medulla itself.

If we turn again to the evidence to be obtained from human pathology, we must realize that the interpretation of observations based on post-mortem material is necessarily much more difficult, and the results must be expected to be less uniform. We cannot study on this material the results of bacterial infections as such. The material which is available is the result of bacterial infections, which were either so virulent and acute that they led to the death of the patient uncomplicated by other factors, or in which the death of the patient was due to some accident or complication, such as a hæmorrhage, nervous paralysis in diphtheria, thrombosis and embolism, etc. In the latter group the accident of death has interrupted the effect of the infection itself on the adrenal or thyroid glands, so that this group corresponds to the intermediate stages of bacterial infections where the animal has been killed before the infection has run its course. For this reason material from acute infections which have led rapidly to a fatal issue are of special value as being capable of a simple interpretation. But one must bear in mind that such material shows only the results which are common to bacterial infections of great virulence, and one must guard against attributing one's conclusions to any specific bacterial infection.

There is the further difficulty that the medulla of the suprarenal gland is so particularly sensitive to post-mortem changes, that unless the gland has been obtained in the first few hours after death it is impossible to draw reliable conclusions from the histological condition or the adrenalin content of the medullary cells. The data concerning the load of the normal human medulla vary, moreover, within very wide limits. We must conclude either that post-mortem changes affect the adrenalin content to an even greater extent than is at present admitted, or that in man the size of the medulla and its load of adrenalin is subject to great variations.

Very extensive and careful series of observations on the human adrenal have been made by Goldzieher (1911), T. R. Elliott (1914), and by Weltmann (1913). Goldzieher directed his attention to the hyperæmia and to the load of adrenalin, which he estimated by a quantitative determination of the adrenalin content.

Elliott's observations show that the cortical lipoid disappears in acute infections if death occurs as the direct result of an acute febrile infection such as measles, scarlet fever, tuberculosis, diphtheria, malignant endocarditis or pneumonia. But in chronic tuberculosis, or in pneumonia of the asthenic type, or in diphtheria when death results from asphyxia or from paralysis of the diaphragm, considerable amounts of lipoids are still present in the cortex. The load of adrenalin is diminished to a varying extent in these conditions, but not to the same extent as the load of lipoid in the cortex. Nor is there any parallelism between the two. Goldzieher, whose observations are based on 4000 autopsies, also found a considerable reduction in the load of adrenalin. He gives an average of 1·5 mg. adrenalin in septic infections as compared with 4 mg. in the gland of the normal adult.

Another indication of a selective participation of the gland in bacterial infections is the greatly increased hyperæmia of the gland. Goldzieher refers to the frequent occurrence of such pathological changes in the adrenals in septic infections as congestion with or without hæmorrhages, thrombosis, necrosis, etc. He states, in fact, that such changes are not the exception, but are a constant occurrence in septic infections, and contrasts this frequency with the rarity of such changes in other organs. According to Symmers (1919), intense injection of the suprarenals is a common post-mortem lesion in influenza, and may be sometimes associated with hæmorrhagic extravasation into the gland. The evidence of other authors concerning the occurrence of adrenal hæmorrhages in the course of acute bacterial infection resulting from the intense congestion of the gland in these conditions has already been dealt with (see p. 98).

The evidence of human pathology confirms, therefore, the results of experimental observation in showing that many bacterial infections produce profound changes in the adrenal glands. The results of our experimental observations enable us to interpret these changes. The intense hyperæmia indicates an increased secretory activity of the gland. The diminution in the load of adrenalin does not in itself necessarily mean an exhaustion of the gland as many writers have supposed. The load of adrenalin represents the balance between the rate at which adrenalin is formed and the rate at which it is secreted. A gland may therefore continue to secrete adrenalin actively though its load is greatly reduced, provided that the new formation of adrenalin is not impaired. We have given reasons for our view that this—the rapid new formation of adrenalin—is dependent upon the functional integrity of the cortex. We do not yet know whether the cortical lipoid is directly concerned in this process, or whether its presence in the resting gland and the changes in its distribution when the adrenal gland is stimulated to activity are merely a visible expression of the functional state of the cortical cells. Since we have seen that the cortical lipoid disappears when we impose an inhibition upon adrenal activity by exposing an animal to a hot environment for long periods, we may look upon this disappearance of the cortical lipoid as indicating a functional inhibition of the cortex. The disappearance of the cortical lipoid as a specific result of bacterial infections of such virulence that they lead rapidly to a fatal issue is capable of the same interpretation. But it must be understood that this inhibition of the cortical activity is attributed, not to bacterial infections as such, but only to bacterial infection of outstanding virulence.

T. R. Elliott (1914) has stated that "the load of lipoid is not directly related to that of adrenalin." Our observations agree with this statement in the sense that a medulla may be depleted of adrenalin while the cortex may be either filled with lipoid or empty of it. But there is this important functional relationship—that so long as the cortex is full of lipoid a depleted medulla may refill itself rapidly with adrenalin, and may in fact be still secreting adrenalin as fast as it can form it. In the absence of lipoid the new formation of adrenalin is impaired, and the condition of such a gland may be described as being in a state of exhaustion.

THYROID GLAND IN BACTERIAL INFECTIONS.

The thyroid gland also shows changes in bacterial infections. Roger and Garnier (1898, 1899) appear to have been the first to point this out, and their observations have been confirmed by a number of authors (Toni, 1900; Kashiwamura, 1901); especially by de Quervain (1906) and his collaborators (Sarband, 1906). Quite recently McCarrison (1917) has again emphasized the effect of bacterial toxins on the thyroid gland. The toxins of intestinal worms (*Ascaris, Tænia*) also produce an effect on the thyroid as they do on the adrenal glands (Bedson, 1913).

The changes which have been observed in these pathological conditions are hyperæmia, desquamation of epithelium and disappearance of colloid. There is reported also a diminution in the iodine content of the gland (Aschenbacher, 1906). Recently Fellenberg (1923) has observed an increased iodine excretion in fever. We may add to this from a study of our own pathological material that occasionally small hæmorrhages occur into the alveoli, so that the "ghosts" of red corpuscles can occasionally be seen lying in the colloid. There has been some discussion about the interpretation which is to be placed upon these histological changes. Our experimental observations show that they indicate a hyperactivity of the thyroid gland—a view which confirms Roger and Garnier. A comparison of Fig. 6, which represents a human thyroid from a case of meningitis with a prolonged severe pyrexia, with Fig. 5, from a rat after the injection of T.H.N., shows an almost identical appearance. There are also very definite and distinct changes in the mitochondria and the Golgi apparatus in the cells of the thyroid gland in different stages of activity (Cramer and Ludford, 1926). But since these cytological changes can be detected only in tissues fixed immediately after death, these criteria cannot be applied to human post-mortem material.

Not all bacterial infections possess this stimulating action on the thyroid to the same degree. According to some authors, scarlet fever, typhoid fever and tuberculosis in their acute forms have a particularly "thyreotonic" effect. Fig. 3 is a drawing of the thyroid gland of a boy, aged 4, who died from miliary tuberculosis. For the last two months he had shown a hectic temperature, and he died in hyperpyrexia (104·5° F.). The gland was deeply congested. The greater part of the section consists of alveoli free from colloid and lined by proliferating epithelium, giving a picture similar to that seen sometimes in exophthalmic goitre. In some parts of the section the epithelial proliferation together with the congestion produced on appearance which made

it difficult to identify the tissue as being thyroid gland. The field drawn has been selected so as to show a few alveoli of normal size and appearance in contact with others exhibiting loss of colloid and epithelial proliferation. McCarrison holds that the toxins of these organisms whose normal habitat is the intestinal canal, such as *B. coli*, dysentery bacilli and cholera bacilli, exercise a particularly profound effect on the thyroid. An interesting observation of comparative pathology bearing on the participation of the thyroid gland in the fever accompanying bacterial infections has been recorded by J. A. Murray (1918). On the basis of a systematic examination of the thyroid gland of warm- and cold-blooded animals which had died in the Zoological Gardens as the result of severe infections he reports as follows: "In warm-blooded animals dying under these conditions extreme congestion of the whole gland is practically constant. Nothing of the kind has been encountered in the reptiles examined, although a large proportion presented severe septicæmic conditions after death. The result is unfavourable to the view that the thyroid plays a part of a neutralizer of toxic substances in the body. It is in much better harmony with the view that the changes in the thyroid in these conditions are the expression of its participation in the heat-regulating mechanism of the body. In poikilothermic animals one would expect these changes to be absent."

In warm-blooded animals the effect of bacterial infections may lead to a functional exhaustion of the thyroid, or there may be organic changes, such as sclerosis, which Roger and Garnier observed after tubercular infection. It is well known that acute infections are sometimes followed by symptoms which may not unreasonably be attributed to disturbances of the thyroid or adrenal function. The stimulation produced by the toxins may develop into a Graves's disease. Cases of Graves's disease have been reported as following upon influenza, typhoid fever and articular rheumatism (Falta, 1913). Or the function of the gland may be impaired as the result of exhaustion. Obesity after recovery from typhoid or from tubercular infection, the falling out of hair and the general asthenia and mental depression after influenza are examples. Even myxœdema and Addison's disease have been known to develop after acute infections. Fulchiero (1923) has reported three cases of malaria having all the classical symptoms of Addison's disease. Sergent and Oury (1923) describe a case of adrenal insufficiency following upon typhoid fever.

Human and experimental pathology furnish evidence, therefore, that in many bacterial infections the thyroid and adrenal glands are strongly affected, particularly in the acute types of infections, such as malaria and influenza, which are accompanied by the greatest disturbances of the heat-regulating mechanism.

THERAPEUTIC APPLICATIONS: SIGNIFICANCE OF FEVER.

This conclusion suggests therapeutic application. In those infections which, like diphtheria, have a particularly adrenalotoxic effect, and in which the temperature is normal or subnormal, it should be of special importance to keep the patient warm so as to relieve the adrenal from the strain of heat regulation. In the case of gas gangrene it could be shown experimentally (Bullock (Gye) and Cramer, 1919), that exposure to cold could transform a

non-lethal dose into a lethal dose. The injection of adrenalin ought to be of special value in acute bacterial infections where there is a sudden failure of the circulation. This is usually ascribed to heart failure, frequently without any pathological evidence. It may be due to the exhaustion of the suprarenals. In a paper published seventeen years ago, John (1909) claimed to have obtained excellent results by the intravenous injection of adrenalin in diphtheria. It may be equally indicated in other acute infections. And if the post-influenzal depression is an expression of an exhausted thyroid adrenal apparatus, it ought to be possible to counteract it by the administration of adrenalin or of thyroid gland. The mode of action of these substances suggests the use of adrenalin in acute conditions, especially in acute failure of the circulation, while thyroid administration is indicated in convalescence after a severe infection, such as influenza for instance. In the only two cases in which I have been able to test the effect of thyroid administration to relieve post-influenzal depression the result fulfilled the expectation. Clemente (1920) records two cases of post-influenzal asthenia in which rapid improvement followed administration of suprarenal extract. The manic-depressive psychosis following influenza has also been attributed to adrenal insufficiency: the same treatment appears indicated.

It is fairly generally agreed to look upon the fever accompanying bacterial infections as a defensive reaction of the organism, in which the rise in temperature is the essential factor. We see now that in many of these fevers there is, in addition to the rise in temperature, yet another factor which may be operative, namely, the pouring into the blood of the thyroid and adrenal hormones and the resultant general sympathetic stimulation. Further investigations will have to show whether these latter factors represent an essential part of the mechanism of defence against bacterial infection, and whether the fever is perhaps a less essential feature than has been believed.

The fact that many bacterial toxins stimulate the thyroid adrenal apparatus illustrates perhaps the curious and as yet unexplained phenomenon, that in some infections the injection of non-specific vaccine and even of proteins may sometimes have a striking therapeutic effect. The effect of the injection of these substances is to produce a typical rigor. The rationale of this so-called non-specific vaccine therapy and protein therapy would be that it elicits an increased functional activity from the thyroid-adrenal apparatus—in fact sympathetic fever, which is one of the normal reactions of the organism against bacterial infection.

The intense sympathetic stimulation which is present in the type of bacterial infection accompanied by a rigor might also explain two characteristic effects: the characteristic constipation could be explained by the inhibition of intestinal movements from sympathetic stimulation; the pulmonary œdema and congestion would also necessarily follow as a result of prolonged sympathetic stimulation. We can readily understand how in such a virulent general infection as influenza the lungs, becoming congested, offer a ready soil for a localized infection, so that a pneumonia develops. It has long been a puzzling feature of influenza epidemics that the disease tends to be more fatal to vigorous individuals. In these the adrenals react most strongly to the stimulating action of the influenza toxin, just as experimentally well

fed and strong animals react most powerfully to the stimulating action of tetrahydronaphthylamin.

SUMMARY.

In previous papers evidence has been adduced to show that the thyroid and adrenal glands form a humoral apparatus concerned in the heat regulation of the body. For the purpose of this paper the conception may be summarized by stating that increased activity of these glands increases heat-production and diminishes heat-loss, so that fever is produced.

In this paper it is shown that an aseptic fever of this kind is also the result of certain pathological conditions involving hyperactivity of these two glands. Examples are Graves's disease and adrenal hæmorrhage. Clinically these conditions closely resemble bacterial infections, and are frequently mistaken for such. Adrenal hæmorrhage in particular is hardly, if ever, diagnosed during life, and usually mistaken for an acute bacterial infection. It is probable that many of the so-called pyrexias of unknown origin are due to disturbances of these two glands.

The similarity between these aseptic fevers and those produced by many microbic infections finds its explanation in the fact that they have the same physiological basis: the adrenal glands and, to a less extent, the thyroid gland are particularly susceptible to the action of many bacterial toxins. Some, for instance diphtheria toxin and the toxins of gas gangrene, have a predominantly toxic action and rapidly exhaust these glands—"adrenalotoxic" and "thyreotoxic" effects. Others have a stimulating action—"adrenalotonic" and "thyreotonic" effects, although in excessive doses they may also exhaust the glands through over-stimulation. Adrenalotonic and thyreotonic actions are accompanied by fever, adrenolotoxic and thyreotoxic actions by a subnormal temperature. The adrenalotoxic effect plays an important part in determining the lethal issue of many microbic infections. It is pointed out that in so far as fever is a defensive reaction against infections, the essential mechanism of defence may be the secretion of the thyroid and adrenal hormones, and the resultant general sympathetic stimulation produced by the stimulating action of microbic toxins rather than the rise in temperature by itself.

The conception of the thyroid adrenal apparatus as a mechanism concerned in controlling body temperature renders it possible to correlate a number of pathological and clinical observations the significance of which has hitherto been obscure: the endocrine origin of pyrexias of certain diseases of the thyroid and adrenal glands and of acute infections, the symptomatology of adrenal hæmorrhage and its prevalence in infants, and the part played by bacterial infections in determining the onset of diseases of the thyroid and adrenal glands.

Some therapeutic applications of this conception are discussed, and it is suggested that it affords an explanation for the non-specific vaccine and protein therapy.

It is a pleasant duty to acknowledge my indebtedness to Prof. E. H. Kettle, Dr. Th. W. P. Lawrence, Dr. D. Paterson and Sir Bernard Spilsbury for their kindness in allowing me facilities for the collection of post-mortem material.

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DESCRIPTION OF PLATES.

FIG. 1.—Thyroid of mouse exposed to cold for 3 hours. All animals were killed by coal-gas. The figure shows the congestion of intra-alveolar capillaries, raising the epithelial cells from the basement membrane and producing desquamation. Formol saline fixation. × 720.

FIG. 2.– Thyroid of mouse showing dilatation of intra-alveolar capillaries and hæmorrhage into an alveolus. The mitochondria are stained. Schridde fixation. × 1640.

FIG. 3.—Thyroid of rat kept for 10 days in cold room at about 4° C. Temperature and weight of animal had remained normal, and no macroscopic lesions were found when the animal was killed with coal-gas. The figure shows a few alveoli collapsed and free from colloid, but retaining their normal appearance. Many alveoli, however, as the result of desquamation of epithelium and hæmorrhages into the alveoli, have lost their typical structure. Schridde fixation. × 280.

FIG. 4.—Thyroid of another rat kept for 14 days in cold room. Weight and temperature normal when killed. No macroscopic lesions found post mortem. The figure shows loss of colloid, small hæmorrhages and recent desquamation of a few cells, in which the mitochondria are still stained. The remaining colloid has a watery consistency, and has lost its staining affinity for Heidenhain's hæmatoxylin which it exhibits in the resting gland. Schridde fixation. × 760.

FIG. 5.—Thyroid gland of a rat which died with pulmonary hæmorrhage 2 hours after injection of 20 mg. tetrahydronaphthylamin. The figure shows intense vascular engorgement of both inter-alveolar and intra-alveolar capillaries, desquamation of alveolar epithelium, and partial disappearance of colloid. Zenker fixation. × 250. (Reprinted, by permission, from the *Sixth Scientific Report of the Imperial Cancer Research Fund.*)

FIG. 6.–Human thyroid gland. Death from meningitis after prolonged severe pyrexia. The figure shows congestion and hæmorrhages into the alveoli, desquamation of epithelium and complete disappearance of colloid. Red blood-corpuscles are represented in black. Formol-Müller fixation, followed by osmic acid. × 360. (Reprinted, by permission, from the *Sixth Scientific Report of the Imperial Cancer Research Fund.*)

FIG. 7.—Human thyroid gland. Death from miliary tuberculosis in hyperpyrexia (104·5° F.) after prolonged pyrexia. Gland shows intense congestion, and in many alveoli epithelial proliferation and loss of colloid. The field has been selected so as to show alveoli exhibiting this change, together with alveoli fairly normal as regards appearance of epithelium and presence of colloid.

FIG. 1.

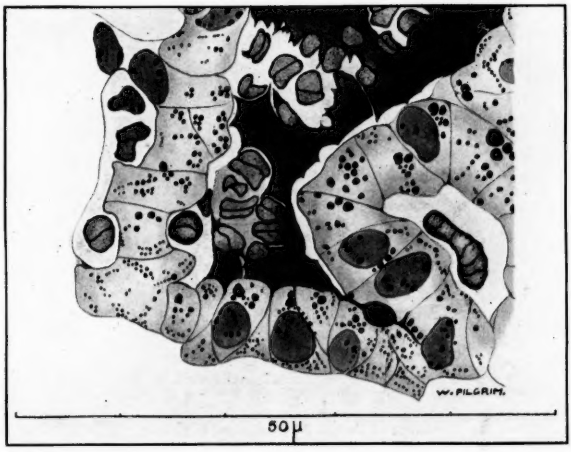

FIG. 2.

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FIG. 3.

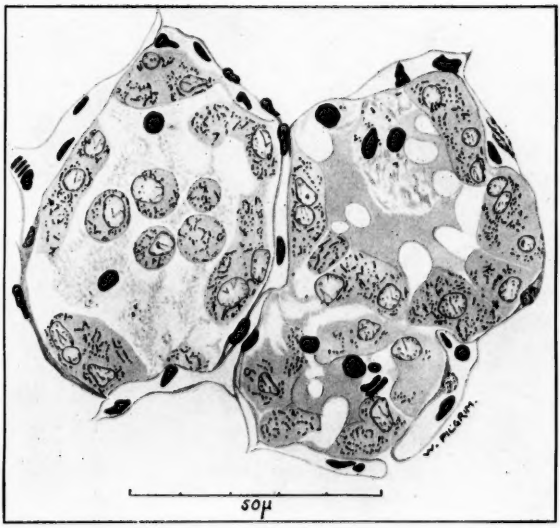

FIG. 4.

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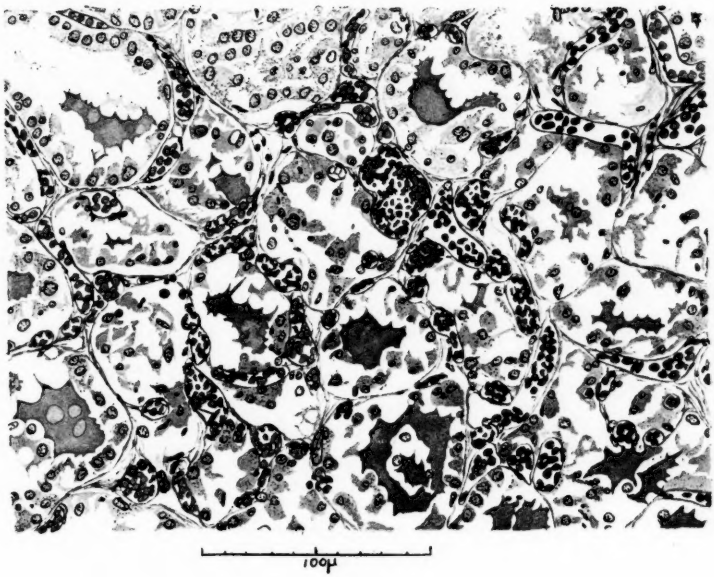

FIG. 5.

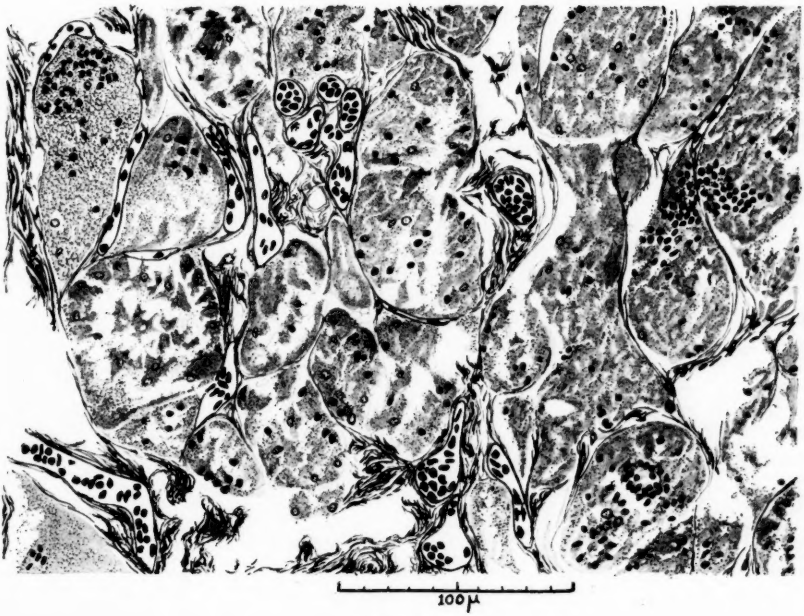

FIG. 6.

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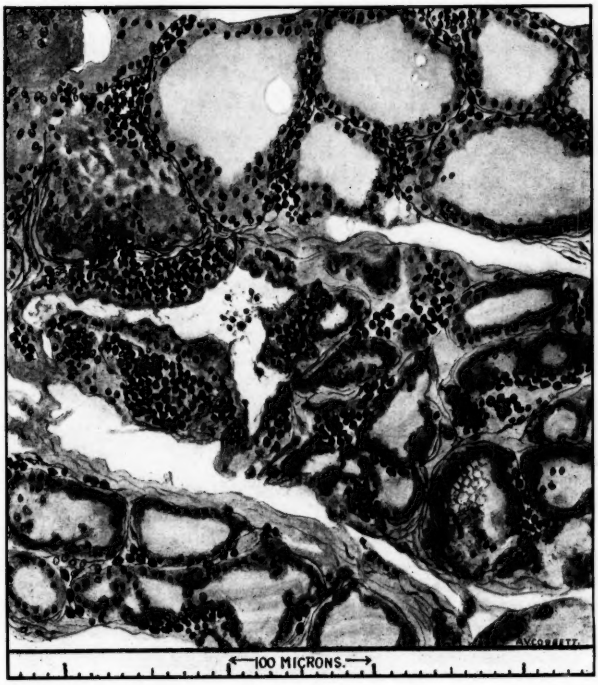

FIG. 7.

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DUODENAL REGURGITATION AND THE CONTROL OF THE PYLORUS.

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That regurgitation is a regular occurrence in normal gastric digestion has been abundantly proved by many recent workers. Boldyreff (1914) placed hydrochloric acid in the stomachs of dogs, and found that the acidity fell rapidly to neutral in from one to one and a half hours by the regurgitation of the mixed juices of the duodenum. Bolton and Goodhart (1922) showed that the variations in gastric acidity during a test-meal were due to variations in the amount of regurgitated fluid rather than to variations in gastric secretion. Apperly (1923) reached the same conclusion using different methods, and showed (1924) that regurgitation was apparently controlled by forces arising within the jejunum. The present paper represents a further inquiry into the nature of the *forces* controlling it, and their bearing on pyloric control.

METHODS.

Experiments were made on normal individuals and on gastric cases before and after operation. The general method of the experiments was the fractional test-meal. The meals used consisted of—

(1) An ordinary salt-free meal of thin gruel of 400 c.c. volume.

(2) As in (1) above, with the addition of hydrochloric acid to make up varying strengths of from 30° to 80° of acid (100° equals $\frac{N}{10}$ solution).*

(3) As in (1) above, with the addition of sodium bicarbonate sufficient to make strengths of from 58° to 116° in different cases.

(4) As in (1), with the addition of sodium chloride of strengths of from 57° to 170° in different cases.

On different days fairly close together these meals were given to each of four healthy students, but in a different order to each man, in order to eliminate any psychic factors attending the first experience of swallowing the Rehfuss tube. The total and free acidity and the total chlorides were estimated in the fasting contents and in each fraction. A similar procedure was followed in the case of patients.

Gorham's criticism (1921), that even with fluid test-meals the gastric contents are not a uniform mixture and that the samples withdrawn are not representative, has been met by exercising great care in mixing the contents before withdrawal by drawing the piston of the syringe to and fro six or seven times. Total and free acidity were determined by titration with $\frac{N}{10}$ alkali, using

* In this nomenclature therefore, if 100° = 0·1 N. solution, 30° = 0·03 N. acid and 80° = 0·08 N. acid. Similarly, for $NaHCO_3$ 58° = 0·058 N. $NaHCO_3$, etc.

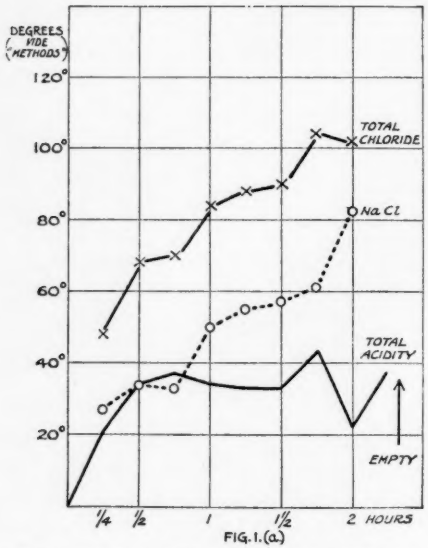

FIG. I. (a.)

S. R—, æt. 32. Ordinary test-meal. 26/3/24.

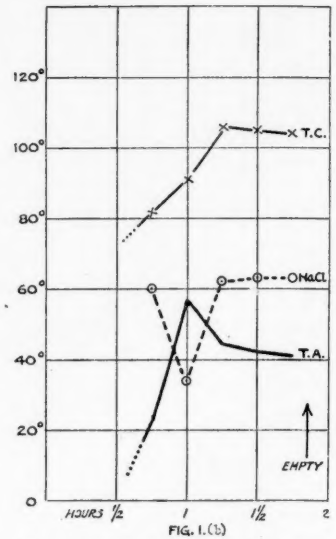

FIG. I. (b.)

S. R—. Test-meal plus sod. bicarb., gr. xxx. 1/4/24.

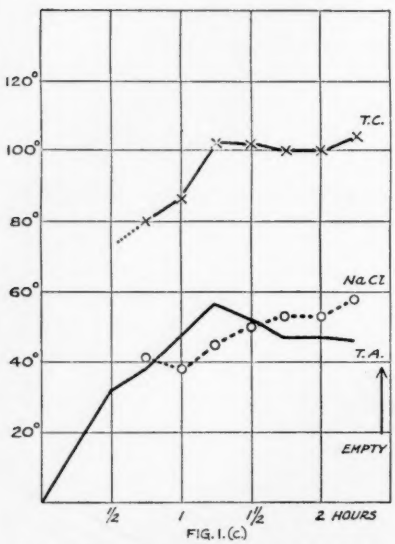

FIG. I. (c.)

S. R—. Test-meal plus NaCl, 1·34 gm. 8/4/24.

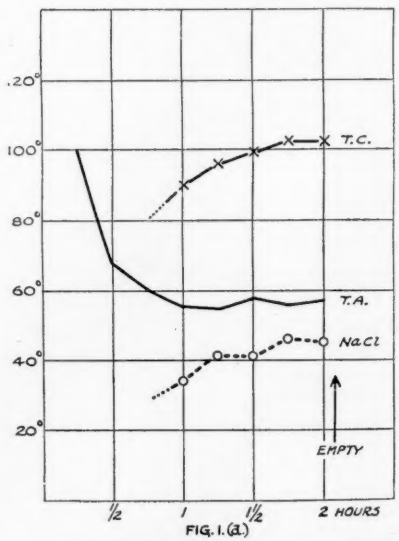

FIG. I. (d.)

S. R—. Test-meal plus 4 c.c. of 10·68 per cent. HCl. 27/3/24.

phenolphthalein and dimethylamidoazobenzol as indicators. Total chlorides were determined by Volhard's method, and the proportion of inorganic chloride present by difference between total chloride and total acidity.

RESULTS.

Fig. 1 shows a typical series of curves from a normal individual. In all cases the total chloride reached a certain definite level (referred to hereafter as the "chloride point") before emptying occurred. This level was different in different individuals, and varied between 80° and 130°. Operative procedures do not appear to affect the final chloride level, as was well illustrated in a patient upon whom tests were performed after gastro-enterostomy, after this operation had been undone, immediately after hemi-gastrectomy, and again a few weeks later (Fig. 2). The fasting contents also have a total chloride strength equal to the "chloride point" for that individual. Generally speaking, individuals with a low chloride point empty rapidly, while those in whom the chloride point is high empty slowly, although in pathological cases there are exceptions to this rule. The average value of the chloride point in a series of individuals has approximately the same value as the average plasma chloride, but varies over much wider limits. Finally, if the inorganic salt strength of the gastric contents is augmented by the addition of sodium chloride or bicarbonate to the meal, the chloride point is unaffected but is reached earlier, with consequent shortening of the emptying time, and the total acidity rises to a higher point than normally.

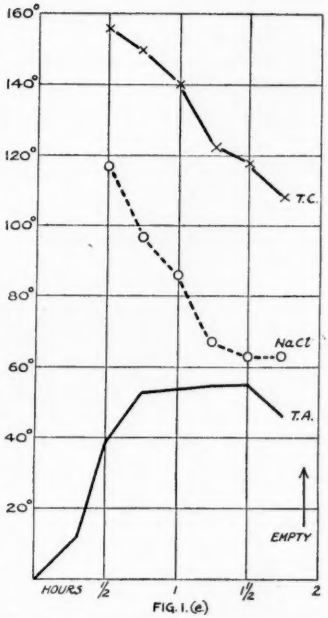

FIG. 1.(e)

S. R—. Test-meal plus 2·39 gm. NaCl. 10/4/24.

These observations suggest that one of the main functions of the stomach is to bring its food contents up to a certain salt strength which will be acceptable to the intestine.

Certain phenomena recorded by other observers find a ready explanation in this hypothesis. Thus Spencer, Meyer, Rehfuss and Hawk (1916) found that 1 per cent. sodium bicarbonate (120°), added to a test-meal, hastens gastric evacuation, and, as in my experiments, that in most cases the acidity was actually raised after the preliminary neutralization. Miller, Bergeim, Rehfuss and Hawk (1920) have shown that 100 gm. of sugar in 40 per cent. and 60 per cent. solutions depress gastric secretion and delay gastric evacuation. In this case we are dealing with a solution of high osmotic value which can only be corrected by much regurgitation of duodenal fluids and salivation, with consequent dilution of acid and delayed emptying. 10 gm. of sugar in 4 per cent. and 6 per cent. solution had no effect, since in these strengths the solution is only slightly hypertonic. Jona (1911) has pointed out that milk is isotonic

with blood, and that "of all other fluids admitted to the stomach of man, alcoholic beverages and fruit juices alone are hypertonic." "In no case is a fluid admitted in which the hypertonicity is due to the mineral ingredients alone, except under protest." "The sense of taste protests . . . and it is undoubtedly by this means that the stomach mucosa is shielded from exposure to foods whose osmotic pressure is far above that of the environment to which the cells are normally accustomed." Jona found that the Δ of soups and beef-teas salted to taste varied from 0·543° to 0·568°, which is nearly that of blood, *i. e.* 0·57° to 0·59°.

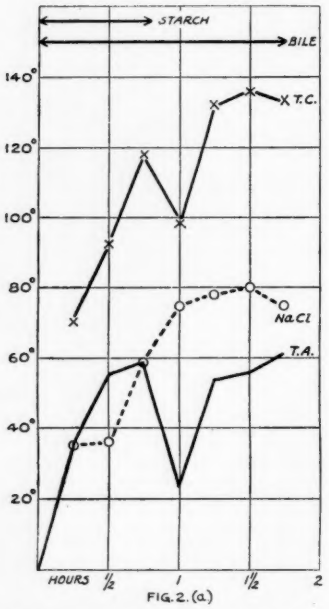

FIG. 2. (a)

H. C—, æt. 40. About a year after gastro-enterostomy for duodenal ulcer. (Test-meal, 18/5/24.)

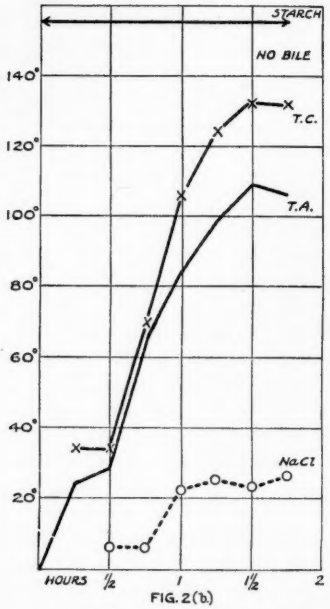

FIG. 2(b)

H. C—. Taken after the unsuccessful gastro-enterostomy had been undone and the parts restored to "normal." 20/6/24.

The Origin and Values of the Salts (chiefly Sodium Chloride) which produce Isotonicity in the Stomach.

In these experiments only the chlorides (of sodium, potassium and calcium) have been measured, but the intestinal contents, and, after complete neutralization, the gastric contents also, contain in addition the phosphates of sodium, potassium and magnesium, which, when added to the 80° to 130° of chloride present, must make up an osmotic pressure equal to about 150°, or 0·9 per cent. sodium chloride, which is isotonic with blood. Hence it would appear, firstly, that the varying chloride curve, though on a lower level than one representing the true osmotic pressure of the contents, can be taken as a

fair indication of the variations in the osmotic pressure of the gastric contents, and secondly, that the stomach retains its contents until a suitable osmotic pressure, roughly isotonic with blood, is reached. If food of less than this value comes in contact with the delicate epithelium of the intestine, it is promptly rejected into the stomach. This is the biological meaning of the reflux force, which is implied in duodenal regurgitation. When finally the food reaches the required osmotic value, it can enter the intestine without stimulating its own rejection, and the stomach empties.

A study of the intimate nature of the forces causing regurgitation leads

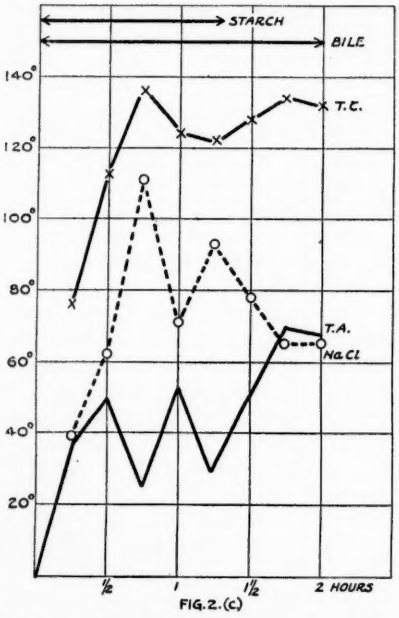

FIG. 2. (c)

H. C—. Taken about a week after a hemi-gastrectomy. About 30/6/24.

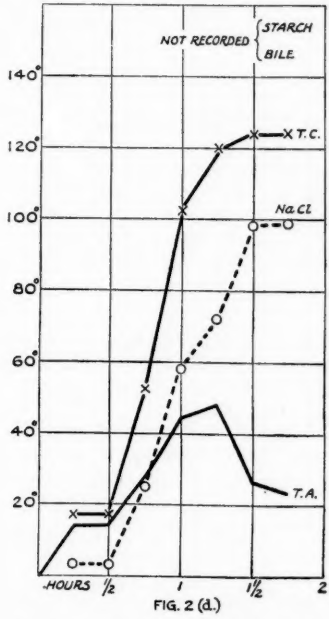

FIG. 2 (d.)

H. C—. Taken about eight weeks after hemi-gastrectomy. 18/4/24.

directly to a consideration of the origin of the salts, chiefly sodium chloride, which produce isotonicity in the duodenal contents. Part of the chlorides are derived from the neutralization of hydrochloric acid by pancreatic alkalis, but the various duodenal juices also contain pre-formed sodium chloride and other salts. Thus the chloride strength of human succus entericus varies from 80° to 100° (Aarons), while that of gall-bladder bile is 120° to 145° (Kröck). In the gall-bladder bile from 21 post-mortem examinations I found the chloride strength to vary from 22° to 112°, with an average of 66°. The alkali strength of pancreatic juice varies from 0·4 per cent. to 0·65 per cent. (80° to 120°) of sodium carbonate, which figures also represent the limits of variation of the "chloride point."

In order to determine the chloride and alkali strengths of the mixed duodenal fluids in human subjects, 250 c.c. of warmed hydrochloric or sulphuric acid of 110° strength, to which glucose or neutral red in known strength had been added, was placed in the stomachs of a number of healthy and dyspeptic individuals by means of the Rehfuss tube. 8 to 10 c.c. fractions were drawn every quarter hour, and in each there were determined (*a*) the acidity, by titration with alkali, (*b*) the total chlorides, by Volhard's methods, and (*c*) the dilution, estimated by the percentage of sugar or dye. For glucose estimations McLean's method was used, while neutral red was determined colorimetrically after the addition of an equal volume of 1 per cent. sodium hydroxide. Ivy and Whitlow (1922) have shown that gastric secretion ceases when mineral acids are placed in the stomach. We may therefore look on the acid curve in the accompanying figure (Fig. 4) as representing the changes in the acid introduced.

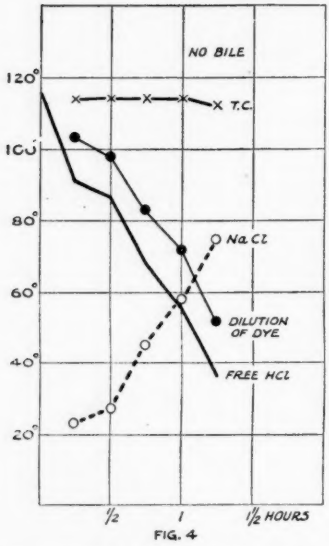

FIG. 4

G. L—, male, æt. 61. After cholecystostomy. Hydrochloric acid test. 11/9/25.

When the results of the estimations were plotted, the following facts were observed: (1) That the fall in acidity is mostly due to dilution by neutral fluids. (2) That a foreign acid placed in the stomach suppresses the secretion of gastric acid, otherwise the acid and dilution curves would cross and reverse their relative positions. Crossing of the curves has never been observed in normal persons, though they have nearly coincided in several cases of old gall-bladder disease in which chronic pancreatitis was probably present. The inorganic chloride curve represents pre-formed sodium chloride secreted as such into the intestine from the glands opening into the duodenum, together with that formed by neutralization of hydrochloric acid introduced. The possibility of secretion of inorganic chloride from the stomach itself must be considered, but against this is the fact that in cases of pyloric obstruction the acidity and total chloride curves in the ordinary test-meal roughly coincide. (3) Knowing the acidity, dilution and sodium chloride in the stomach contents at any moment, we can calculate the absolute strengths of alkali and sodium chloride in the mixed duodenal fluids. For this purpose Prof. J. H. Michell, F.R.S., has suggested the following formula when hydrochloric acid is used in the test: $\frac{k}{K+a} = \frac{G-g}{G} = \frac{H-h}{H+a}$, where k is the sodium chloride strength, h = the acidity, and g = the strength of glucose or neutral red in the gastric contents at the moment considered; H = the original acid strength and G = the original glucose strength placed in the stomach; and K = the sodium chloride, and a = the alkali strength of the mixed duodenal fluids which we want to

know. (When sulphuric acid is used the expression $\frac{k}{K}$ replaces $\frac{k}{K+a}$ of the formula.)

TABLE I.

Case.	Acidity at 1 hour.	Gastric NaCl at 1 hour.	K (duodenal NaCl).	a (duodenal alkali).	Case.	Acidity at 1 hour.	Gastric NaCl at 1 hour.	K (duodenal NaCl).	a (duodenal alkali).
1	—	37	42	—	22	41	50	80	—
2	—	45	40–50	—	23	35	58	98	—
3	20	45	62	—	24	10	38	42	—
4	26	24	31	—	25	70	60	160	—
5	44	50	80	—	26	46	42	72	—
6	46	34	58	—	27	40	44	70	—
7	48	46	82	—	28	34	34	50	—
8	50	46	85	—	29	53	56	108	—
9	52	40	76	—	30	70	20	55	—
10	40	30	47	—	31	56	58	110	42
11	60	36	77	23	32	80	30	94	40
12	27	76	103	7	33	35	55	80	2
13	42	23	42	15	34	21	64	76	8
14	84	46	140	—	35	57	37	66	36
15	44	34	57	—	36	54	28	23	57
16	64	34	70	18	37	46	36	62	—
17	37	28	47	15	38	63	28	65	—
18	22	25	24	10	39	70	64	150	—
19	30	60	73	37	40	40	42	66	—
20	19	34	42	—	41	80	48	150	—
21	90	40	140	—	42	73	41	87	32

In normal cases the value of K varies from 40° to 90°, average about 66°, and that of a is about 15° to 20° at one hour, but great variations of K occur in disease, and are by far the most important cause of variations in gastric acidity. Table I shows the values obtained in 42 cases. In only 14 of these has the full method as described above been carried out, giving the true values of K and a. In the remainder, values of K are approximations calculated on the assumption that $K = k\frac{H}{H-h}$, which, from Mitchell's formula, equals $\frac{H(K+a)}{H+a}$, which, again, as will be seen later, is the value of the duodenal sodium chloride at the moment considered, instead of the average value up to that moment. Actual experiment, however, shows that the values so obtained are correct to within 4° to 6°.

If the values are grouped as in Fig. 3, it will be seen that a rise in the value of K is associated with a rise in both acidity (h) and NaCl (k) values, whereas high values of the duodenal alkali, a, appear to produce a rise in acidity, but no appreciable effect on the sodium chloride curve. These effects of variations in alkali are, however, by no means so definite as the effects of variation in the value of K. Thus various considerations have shown that the values of K and a tend to vary inversely, so that low values of a (as in pancreatic disease) produce a higher acidity curve than normally (Apperly and

Cameron, 1923), instead of the reverse, as we would expect were the value of K to remain constant.

When a volume of 0·4 per cent. hydrochloric acid (110°) is placed in the stomach, the character of the acidity chart (Fig. 4) is obviously determined by the volume and the nature of the returned duodenal fluids. The factor which determines regurgitation is the difference which may exist at any given moment between the "chloride point" for that individual and the sodium chloride strength of the chyme in the duodeno-jejunum. This last factor may be represented as follows: Let a volume, c, of HCl of strength H enter the duodenum, where it elicits a secretion of volume p, with alkali and NaCl strengths of a and K respectively. When neutralization is complete, then $pa = cH$, and the NaCl value will now be $\frac{p\,(a+K)}{c+p}$, which equals $\frac{H\,(a+K)}{a+H}$. It follows, therefore, that where H is greater than K, as in the hydrochloric acid test-meal, there is an increased reflux when the values of K, H or a are diminished, other factors remaining constant. The acidity of the gastric contents, therefore, tends to be diminished in individuals with low values of the chloride or alkali of the mixed duodenal fluids. That rise of K diminishes the reflux and leads to higher acidity was shown in the experiments described in the early part of this paper, when sodium chloride was added to the test-meal (Fig. 1). Sodium bicarbonate, by forming sodium chloride before entering the duodenum, acted in the same way.

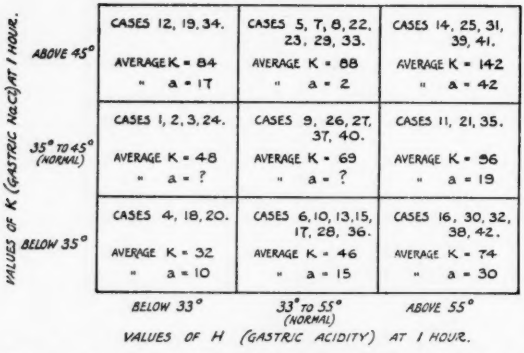
VALUES OF K (GASTRIC NaCl) AT 1 HOUR.
ABOVE 45°
35° TO 45° (NORMAL)
BELOW 35°
CASES 12, 19, 34.
AVERAGE K = 84
" a = 17
CASES 5, 7, 8, 22, 23, 29, 33.
AVERAGE K = 88
" a = 2
CASES 14, 25, 31, 39, 41.
AVERAGE K = 142
" a = 42
CASES 1, 2, 3, 24.
AVERAGE K = 48
" a = ?
CASES 9, 26, 27, 37, 40.
AVERAGE K = 69
" a = ?
CASES 11, 21, 35.
AVERAGE K = 96
" a = 19
CASES 4, 18, 20.
AVERAGE K = 32
" a = 10
CASES 6, 10, 13, 15, 17, 28, 36.
AVERAGE K = 46
" a = 15
CASES 16, 30, 32, 38, 42.
AVERAGE K = 74
" a = 30
BELOW 33°
33° TO 55° (NORMAL)
ABOVE 55°
VALUES OF H (GASTRIC ACIDITY) AT 1 HOUR.

FIG. 3.—A classification of the cases shown in Table I according to their values of h and k (gastric acidity and NaCl respectively) at one hour, and their relationship to duodenal alkali and NaCl strengths (a and K).

Variations in the composition of the duodenal fluids are also of obvious importance. From inspection of Michell's formula it may be readily deduced that, if the dilution remains constant, (a) an increase in the value of K causes a corresponding increase in the value of k (inorganic chloride), and (b) a rise in the value of a diminishes the acidity, but increases k. The above theoretical considerations agree well with the experimental results.

The Mechanism of Pyloric Control.

These considerations point to variations in the reflux force and consequent changes in gastric acidity as being dependent upon the values of the alkali and sodium chloride in the mixed duodenal fluids, of which the latter appears to be the more important. Apart from some tabetics and other patients in whom

reflex pyloric spasm is present, the neuro-muscular mechanism of the pylorus appears to play a secondary rather than a primary part in the regulation of gastro-duodenal flux and reflux. The view that I have put forward is an extension of Cannon's theory (1911) of acid control of the pylorus. Acid in the duodenum produces pyloric closure, but its rate of neutralization depends on the amount of regurgitation, which again depends on the alkali, and particularly the sodium chloride values of the duodenal fluids. The control of the pylorus, therefore, ultimately depends on the salt values of the duodenal fluids, which therefore determine the acidity and the rate of evacuation of the stomach. If we know the values of K and a we can, in the great majority of cases, approximately predict the resulting acidity curve when 250 c.c. of 0·4 per cent. hydrochloric acid are placed in the stomach. The fact that McClure, Reynolds and Schwartz (1920) failed to produce pyloric closure by injecting hydrochloric acid into the duodenum by means of the duodenal tube is readily explained by the immediate reflux and neutralization of the acid by alkali. When acid and alkali mix, half the ions present are destroyed to form water, with a fall in the osmotic value of the mixture and consequent reflux. Reference has already been made to the observations of Spencer, Meyer, Rehfuss and Hawk (1916) that when 120°, or 1 per cent. solution of sodium bicarbonate (a strength close to the value of the "chloride point"), was added to a test-meal, the evacuation time was diminished, but when 5 per cent. of the salt was added the time was longer than normal. In some cases the stomach emptied while the contents were still alkaline, but not till the alkalinity was reduced to about 100°, or the average value of the "chloride point."

SUMMARY.

(1) Before the intestine will accept chyme from the stomach, the chyme must be of a certain salt strength or osmotic value. Any strength of contents above or below this value is potentially injurious to the intestine, and is therefore rejected by the latter into the stomach, where it remains till such time as it has, by the addition of secreted hydrochloric acid, and of sodium chloride to the rejected contents, or by dilution, attained the correct salt strength. The stomach then empties.

(2) The "chloride value" to which the chyme must attain is a constant for each individual, but varies in different individuals from 80° to 130°, with an average of about 100°, or the average chloride strength of plasma ($100° = \frac{N}{10}$).

(3) The strengths of the acid, alkali and salts entering the duodeno-jejunum determine the amount of regurgitation, which in turn regulates the rate of neutralization of duodenal acid and therefore the control of the pylorus. This thesis is therefore an extension of Cannon's theory of acid control of the pylorus.

I wish to record my gratitude to Prof. J. H. Michell, F.R.S., to Dr. C. H. Kellaway, Director of the Walter and Eliza Hall Institute of Research, and to Associate Prof. W. J. Young, of the Biochemical Department, University of

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SERUM MAGNESIUM IN RELATION TO MENSTRUATION: WITH A NOTE ON THE CALCIUM.

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As far back as 1894 Schrader found a retention of nitrogen immediately before and during menstruation. Hafkesbring and Collett (1924) state that there is a rise in basal metabolism before the period, followed (in the absence of pain) by a sharp drop either just before or just after the onset. Earlier works of Blunt and Dye (1921), however, failed to demonstrate any definite variations in basal metabolism. Puxeddu (1924) found an increase in serum protein one or two days before and on the first and second days of the period, amounting to 15–20 per cent. Heilig (1924) states that there is a tendency towards a retention of water and chloride during the first two days, and a marked disturbance of sugar metabolism. Okey and Robb (1925) also found a disturbed sugar metabolism, though the values were sometimes higher and sometimes lower than normal. Further interesting work has been done by Okey (1925), who has shown a rise of uric acid (6 per cent.) before or at the onset of menstruation, though urea and creatinine did not appear to show any

change. She found also an abrupt fall in cholesterol (40–100 per cent.) during a period of 3–10 days before menstruation, with a rise beginning soon after the onset. The work of Klaus (1925) may also be mentioned. He found that the sweat contained about 48 times more cholin immediately before and during the first days of menstruation than was present in the inter-menstrual period.

The present investigations were started during the course of a number of determinations of the magnesium content of serum in a variety of pathological and experimental conditions. So far only 14 cases have been examined, but these show definite types of variations.

Method.

The method employed was based on that of Hammett and Adams (1922). The supernatant fluid from the calcium precipitate obtained from 2 c.c. of serum by Kramer and Tisdall's method (1924) is poured off into a clean dry tube, and 4 c.c. of this (equivalent to 1·6 c.c. of serum) is accurately measured into a 15 c.c. centrifuge tube. The precipitation and washing is carried out as described by the above authors. The ammonia is evaporated off by warming the tube in a water-bath for a few minutes; the precipitate is then dissolved in 5 c.c. 5 per cent. H_2SO_4, transferred to a 25 c.c. volumetric flask, and the tube washed out into the flask by means of about 5 c.c. distilled water. The procedure is then the same as that adopted by Hammett and Adams, except that 1 c.c. of a 20 per cent. sulphite solution is used instead of the carbonate-sulphite mixture, and 5 c.c. of 5 per cent. H_2SO_4 is added to the standard flask so that the acidity of the two solutions to be compared is identical. The solutions should be left 30 minutes before reading in the colorimeter.

PERSONS IN THE INTERMENSTRUAL PERIOD.

The normal magnesium content of serum is frequently stated as 2–4 mgm. per 100 c.c., but this is far too wide a range; only grossly abnormal cases fall outside these values. During the present work on magnesium over 250 sera from about 200 individuals have been examined, with the result that all normals have been found to be between the values of 2·24–2·58 mgm. per 100 c.c. serum. Notable exceptions were met with during the exceptionally hot weather of last summer, when the normal values were found to be increased—ranging from 2·40–2·70 mgm. per 100 c.c.

This latter fact had to be taken into consideration in all cases examined during the summer, and may be compared with the seasonal variations in blood phosphate found by Havard and Reay (1926). It has been shown elsewhere (Watchorn, 1925) that the value is apparently very constant in each individual during health. As the normal range is so limited and constant, small variations become of greater significance than would otherwise be the case. In the large number of pathological cases investigated it was only very rarely found that the serum magnesium was *lower* than normal, and was, with a single exception (a value of 1·9 mgm. per cent. in a case of pituitary tumour with acromegaly), never below 2·1 mgm. per cent. This fact lends greater interest and significance to some of the findings in menstruation, described below.

Menstruation.

Of the 14 cases examined, only one had at any time sought medical aid for abnormal symptoms (Case 9), although one other had been very ill each month (Case 3). There was no excessive loss in any of the women. An endeavour was made to obtain blood some twelve hours before the onset of the menstrual flow, as other researches above referred to, and the fact that malaise (as distinct from *pain*) is generally most pronounced at this time, led one to expect that any change in magnesium would be then more evident than after the establishment of the flow. A normal sample of blood was taken from each subject about a fortnight before the expected start of the period, and when possible further samples were taken during the period, though the fact of having to obtain blood from a vein was found to be a serious limiting factor in the investigations, volunteers naturally not being willing to submit to more than one or two punctures. A marked nervous aversion to the operation was felt by nearly all the women at about the menstrual period, even in those in whom it was entirely absent at other times.

Of the 14 cases, 5 showed no change whatever and will not be further considered till later. The remainder will be described in detail. All estimations were done in duplicate.

Case 1.—Regular, no pain, but marked faintness, dizziness, throbbing headache and general malaise for several days before menstruating.

Normal value (mean of several determinations, ranging from 2·38–2·58 mgm. per 100 c.c.)=2·55 mgm. per 100 c.c.

12 hours before onset = 1·97 mgm. per 100 c.c.
4 ,, after ,, = 2·19 ,, ,,

Several months later this subject was again examined. There was a high nitial value of magnesium owing to a recent infection.

10 days before onset = 3·15 mgm. per 100 c.c.
18 hours ,, ,, = 1·89 ,, ,,
Immediately after onset = 2·23 ,, ,,
3 days ,, ,, = 1·88 ,, ,,
5 ,, ,, ,, = 2·30 ,, ,,

Case 2.—Fairly regular, no pain, but faintness, extreme pallor, headache, etc.
Normal (same figure obtained on two occasions) = 2·69 mgm. per 100 c.c.
Immediately after onset = 2·28 mgm. per 100 c.c.
This was during the hot weather, hence the higher values.

Case 3.—Very irregular. Menstruation accompanied by fainting and intense nausea, sometimes severe pain and vomiting. Always felt and looked very ill for several days before the onset.

Normal = 2·31 mgm. per 100 c.c. (average of 5 determinations, ranging from 2·23–2·38 mgm.).

12 hours before onset = 1·73 mgm. per 100 c.c.
2 ,, after ,, = 2·09 ,, ,,
5th day ,, ,, = 2·41 ,, ,,

There was no pain on this occasion. This subject gradually improved in general health, and nearly a year later was examined again. She was then

menstruating without any of the symptoms mentioned above. Her magnesium on this occasion remained normal.

10 days before onset = 2·31 mgm. per 100 c.c.
1 day „ „ = 2·38 „ „
1 „ after „ = 2·39 „ „

Case 4.—Regular, no pain, always great lassitude, and pallor.

Normal (one determination) = 2·33 mgm. per 100 c.c.
Just after onset of period = 1·88 „ „

Case 5.—Regular, often no symptoms, but on this occasion was feeling faint. Her normal serum magnesium figure was rather low.

Normal = 2·27 mgm. per 100 c.c. (same figure obtained on two occasions).
6 hours after onset = 2·17 mgm. per 100 c.c.

Case 6.—Generally felt very fit, and was examined at one of these periods, when her magnesium showed no change. A few months later she offered to be bled, complaining of nausea, coldness, etc. Her menstrual period started 12 hours later.

Normal (average of 3 determinations during the summer) = 2·67 mgm. per 100 c.c.
12 hours before menstruation = 2·14 mgm. per 100 c.c.

Case 7.—Normal and regular, but generally had pain for first few hours.

Normal (one determination) = 2·46 mgm. per 100 c.c.
6 hours after onset = 2·85 mgm. per 100 c.c.

Case 8.—Was examined during two menstrual periods. The normal (average of two determinations) was 2·34 mgm. per 100 c.c., and 8 hours after the onset; 2·66 mgm. per 100 c.c. there was then slight pain, but otherwise she was feeling very well. Three months later there was a menstruation accompanied by violent pain. Four hours after the onset the serum magnesium had the very high value of 5·02 mgm. per 100 c.c.

Case 9.—Always had severe headache before period, and appeared thoroughly ill. Was bled twice. (*a*) Period due: Abdominal pain and backache. Serum magnesium=2·96 mgm. per 100 c.c. (*b*) Two days later=3·34 mgm. per 100 c.c. There was then severe headache, extreme lassitude and bad abdominal pain. Menstruation started 4 hours later. Unfortunately no normal of this patient was ever obtained for comparison, and as her general health was not good in several ways, the inter-menstrual value may also have been raised. However, this would not alter the fact that there was a pre-menstrual rise of the serum magnesium.

DISCUSSION.

The fourteen cases examined fall into three groups:

(*a*) those in whom there was no change in serum magnesium;
(*b*) those whose serum magnesium showed an increase;
(*c*) those who showed a premenstrual decrease, or decrease a few hours after onset, varying in degree up to 40 per cent.

The women classed under (*a*) menstruated without any previous symptoms, and did not experience anything but slight tiredness (if that) on the first day. Cases 7–9 (Group *b*), however, all showed a rise, in one case 100 per cent. These three women all had pain, and in the writer's opinion the rise in serum magnesium was due to the pain, and had no specific connection with menstruation. This awaits confirmation, but much evidence in support of this has been obtained during the present work in various pathological and experimental conditions. As pointed out above, Hafkesbring and Collett found that pain prevented the pre-menstrual drop in basal metabolism. Group (*c*) (Cases 1–6) were much the most interesting, as their serum showed the decrease in magnesium, so rarely found. All six women complained of *pre*-menstrual malaise, and in each case pain was absent. It is noteworthy also that two women (Cases 6 and 9) were each normal on one occasion when feeling quite fit, whereas they showed variations on other occasions when feeling ill.

In work done on blood in relation to menstruation by other investigators no mention is made of any symptoms. The women are described as "normal," but this merely seems to mean the absence of any definite disease. In the cases above described, decrease in serum magnesium seemed always to correspond to pre-menstrual malaise, and it would be instructive to know whether decreases in other blood constituents were similarly related to symptoms or not. In view of the possible connection between the pituitary and sex-glands at œstrus (Dixon and Marshall, 1924), it is interesting that the only pathological case showing a decreased value comparable to those found in menstruation was one of pituitary tumour. Four cases of hyperthyroidism examined at about the same time were strictly normal as regards magnesium.

EFFECT OF TAKING MAGNESIUM.

Owing to the evidence of decreased magnesium content of the serum accompanied by pre-menstrual malaise obtained in Cases 1–6, it was decided to try the effect, if any, of taking magnesium before menstruation. The subject described as Case 1 was chosen for this, as she had shown the marked decrease on three occasions. It has been shown by the writer and Haldane in unpublished work that large doses of $MgCl_2$ cause a rise in serum magnesium, but such a method of raising the magnesium is not practicable, as apart from some acidosis produced there is intense diarrhœa. In the present case magnesium acetate was chosen, 1 gm. well diluted with water being taken immediately after each meal. 21 gm. in all were taken, extending over a period of six days. No intestinal disturbance was felt till after the last dose, when there was some diarrhœa. After 9 gm. had been taken a severe backache developed, followed by deep-seated pain in the left side of the abdomen, which was exactly similar in type and position to the pain experienced about two years before, during an attack of ovaritis (the right ovary had been removed several years before). There was drowsiness, depression and dyspnœa. Menstruation came on two days after the last dose. Any symptoms due to menstruation were, of course, masked by the apparent ill-effects of the magnesium. The magnesium content of the serum rose from 2·31 to 2·74 mgm. per cent., and was at the latter level just before menstruation started. This raising of the serum value by taking a

magnesium salt appears to be in contrast to the well-known difficulty (amounting almost to an impossibility) of raising the calcium content of the serum by administration of calcium salts. On the other hand, it is by no means certain that this rise was due to the salt taken; the possibility that it was due to the pain and dyspnœa must be borne in mind. In this connection it should be stated that I have found an increase of 50 per cent. in the magnesium of serum in hysterical dyspnœa, and an increase of 18 per cent. in voluntary forced breathing. Haldane (1925) suggests that magnesium has an effect on the respiratory centre and thus causes increased breathing. If so, this would account for the dyspnœa in the present experiment. A urinary analysis was made on a 24-hour collection before the beginning of the experiment, and again on the fifth day. The magnesium excretion rose from 152 to 256 mgm. per 24 hours, so that some of the administered salt must have been absorbed. The calcium excretion by the kidney did not alter, being 214 and 208 mgm. per 24 hours respectively. The administration of magnesium has been stated by various workers (Hart and Steenbock, 1913; Bogert and McKittrich, 1922) to raise the urinary excretion of Ca, but did not appear to do so on the present occasion. An interesting point is that the calcium of the serum decreased from 11·4 to 9·1 mgm. per cent.

Five months later the procedure was repeated. On the third day (*i. e.* after taking 9 gm.) severe dyspnœa again came on. The pulse was 116, and the experimenter felt thoroughly ill. As it was particularly desired to avoid an overdose no more of the salt was taken. The serum mgm. rose from 2·50 to 2·76, and the Ca again decreased 10 per cent.

The backache and apparent ovarian pain again developed, but this time all symptoms rapidly passed off, and 2 days later the experimenter was feeling well again. Menstruation came on 3 days later, and was pleasingly and unusually normal—no vertigo, faintness or nausea being felt. It is, of course, difficult to say how much of these symptoms was mere coincidence, or even the result of suggestion in the second experiment, but they are not without interest, and further work might be worth while along these lines. It is suggested that not more than 1·5 gm. should be taken daily for a period of four or five days preceding the expected menstruation.

CALCIUM IN MENSTRUATION.

In view of Blair Bell's (1908) findings of very low serum calcium preceding menstruation, the opportunity has been taken to test this, but either no change at all was found, or only a small one. For instance, the greatest change was shown by Cases 1, 3 and 4, as follows:

Case 1.	Normal	=11·49 mgm. per 100.	Day before menstruation 10·33 mgm. per 100 c.c.
„ 2.	„	=11·53 „	Day before menstruation 10·78 mgm. per 100 c.c.
„ 4.	„	=10·60 „	Day before menstruation 9·82 mgm. per 100 c.c.

These changes were shown by those women displaying the greatest change in serum magnesium, but even so the decrease is enormously less than that found by Blair Bell, who got values as low as one-fifth normal on the day preceding menstruation.

SUMMARY.

1. Women who had no premenstrual pain or other symptoms showed no change in serum magnesium.

2. There was an increase in the serum magnesium when pain was present, and a decrease when there was premenstrual malaise without pain.

3. Taking magnesium acetate during the week preceding menstruation apparently caused ovarian irritation, but was on one occasion followed by an unusually normal menstrual period.

4. Very little change, or no change at all, was found in the serum calcium.

My thanks are due to Prof. Sir F. G. Hopkins for his interest in this work, and to Dr. J. R. Marrack, of the London Hospital, for kindly supplying me with the pathological sera, and especially to Mr. J. B. S. Haldane for much helpful criticism.

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THE ANTI-COAGULANT ACTION OF HIRUDIN.

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EXPERIMENTAL investigation of the delayed clotting observed in hirudinized plasma has led different workers to opposed conclusions. Thus hirudin has been inferred to be antifibrinogen (Bodong, 1905), antiprothrombin (Arthus, 1920), antithrombin (Gratia, 1920), antikinase (Howell, 1911–12), and antithrombin and antikinase (Mellanby, 1909).

In the present communication the behaviour of hirudin in respect of the coagulation of fibrinogen by thrombin is further studied. The stock solution of hirudin employed contained approximately 1 part of solid in 500 : fibrinogen was represented by citrated human plasma ; and as source of thrombin the venom of *Echis carinatus** was used. In all the experiments made the possibility of interaction of thrombokinase and prothrombin, contained in the solutions employed, has been excluded by citration : only three reacting substances have to be considered, namely, hirudin, fibrinogen and thrombin. The action of hirudin must therefore be assumed to be directed either to (1) fibrinogen or to (2) thrombin.

An attempt was made to discover the direction in which the action of hirudin was exerted by mixing hirudin and thrombin together for some time before adding fibrinogen, or alternatively, by mixing hirudin and fibrinogen before adding thrombin, both types of experiment being compared with controls in which all three components were mixed as nearly as possible simultaneously. These experiments gave no information, however, the action of hirudin being obviously too rapid for the mode of admixture to affect the coagulation period. It was therefore necessary to seek some other line of investigation.

Since the action of hirudin must be exerted upon either fibrinogen or thrombin, the effect of this action would naturally be to diminish the amount of fibrinogen or thrombin, as the case may be, available for producing a coagulum. Retardation of coagulation by hirudin would thus be similar to that brought about by dilution. Now the ultimate effect of dilution of fibrinogen (the amount of thrombin present remaining unaltered) is to cause diminution of the number of fibrils observed when clotting occurs, these becoming shorter and finer, and finally disappearing ; dilution of thrombin, on the contrary (the amount of fibrinogen present remaining unchanged), while also attended by diminution of number, is characterized by increase in length and thickness of the fibrils which make their appearance (Barratt, 1920—in this article illustrations are given). In order to determine whether the action of hirudin is comparable to a dilution effect the experiments given in Table I were made: Experiments 1–3 exhibit the result of dilution of thrombin, Experiments 1, 4 and 5 that of dilution of plasma, and the last three the effect of adding hirudin. The experiments were carried out by adding at room temperature (16°C.–18°C.) 1 part of citrated saline containing thrombin or

* I am indebted to the kindness of Dr. C. J. Martin, Director of the Lister Institute, for a supply of this venom.

TABLE I.

Number of experiment.	Plasma.	Thrombin.	Hirudin.	Onset of coagulation at end of—	Fibrils.		
					Number.	Length.	Thickness.
1	·80	·591 × 10^{-6}	—	8 mins.	Very numerous	12μ to 35μ	·1μ to ·4μ
2	·80	·147 „	—	39 „	Numerous	30μ to 90μ	·2μ to ·7μ
3	·80	·037 „	—	100 „	Moderately numerous	50μ to 200μ	·2μ to ·9μ
1	·80	·591 „	—	8 „	Numerous	12μ to 35μ	·1μ to ·4μ
4	·50	·591 „	—	7·3 „	Very numerous	6μ to 10μ	·1μ to ·2μ
5	·20	·591 „	—	14 „	None seen	—	—
6	·80	·591 „	9·47 × 10^{-6}	9 „	Very numerous	10μ to 25μ	·1μ to ·2μ
7	·80	·591 „	37·9 „	23 „	Numerous	15μ to 35μ	·1μ to ·3μ
8	·80	·591 „	151 „	183 „	Moderately numerous	20μ to 180μ	·2μ to ·1μ

a mixture of thrombin and hirudin to 5 parts of citrated human plasma, undiluted or diluted with citrated saline as indicated in the Table; the second column gives the amount of plasma contained in 1 c.cm. of the mixture, and the third and fourth columns the amounts of thrombin and hirudin respectively. If the last series (Experiments 6–8) is compared with the first two, it will be seen that retardation of coagulation by hirudin resembles that obtained by dilution of thrombin (Experiments 1–3), being attended by marked diminution in number and increase in length and thickness of fibrils; the effect is in striking contrast to that of dilution of fibrinogen (Experiments 1, 4 and 5), characterized by a less marked delay of coagulation and by a diminution in length and thickness of fibrils, which in the last experiment (5) cease to appear. It is obvious, therefore, that the effect of hirudin is to cause disappearance of thrombin, fibrinogen being unaffected. In other words, hirudin is an antithrombin. Some idea of the amount of thrombin disappearing will be obtained by comparing Experiments 6, 7, 8, with 1, 2, 3. The increase of hirudin does not, however, bear a simple proportion to the quantity of thrombin removed; when increasing quantities of hirudin are added the corresponding amounts of thrombin disappearing markedly diminish.

It will be of interest to refer, before concluding this article, to the work of Gratia, who adopted a different method of experiment in order to prove that the action of hirudin was exerted upon thrombin. This observer employed a solution of thrombin which was found to deteriorate in course of time. To a portion of the solution of thrombin hirudin was added in amount sufficient to inhibit its coagulating action upon fibrinogen. It was inferred that when the fraction to which no hirudin had been added had lost its power to coagulate fibrinogen, then in the hirudin fraction the same deterioration of thrombin would also have occurred if hirudin had no action upon thrombin, and thus the anticoagulant action of hirudin would reappear. It was, however, found that the anticoagulant action of hirudin did not reappear, and it was therefore concluded that hirudin neutralized thrombin. This method of proof seems open to the objection that if hirudin were without any direct action upon thrombin, it might nevertheless happen that hirudin or some other constituent in the hirudin solution employed might inhibit the process of deterioration of

thrombin. This possible objection is avoided by the method of comparison with the effect of dilution of fibrinogen and thrombin respectively given above.

SUMMARY.

Experiments made to determine the effect of hirudin upon fibril formation in citrated fibrinogen solution (blood-plasma) show that hirudin: (1) acts upon thrombin, which becomes diminished in amount: and (2) is without action upon fibrinogen.

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THE EFFECT OF CLOTTING AND OF SODIUM CITRATE ON THE BACTERICIDAL POWER OF THE BLOOD.

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THESE experiments were undertaken to investigate the effects of sodium citrate and of coagulation on the bactericidal power of the blood. Colebrook and Storer (1924) have stated that citrate inhibits bactericidal power, and that this inhibition is far more marked in freshly-shed blood than in serum.

In the first series of experiments about to be described the relative effects of citrate and saline were compared.

First Series of Experiments.

Blood was taken from the vein of a normal human subject and defibrinated. The defibrinated blood was then divided into two equal parts, and to one part was added 1/20 of its bulk of 0·9% saline, and to the other part was added 1/20 of its bulk of 0·25% sodium citrate.

The two samples were then compared with respect to their bactericidal properties for staphylococci. This was done by "inculturing" in slide cells following the method described by Wright, Colebrook and Storer (1923).

A suitable dilution of a twenty-four hours old culture of staphylococcus in broth was made, and 2·5 c.mm. of this dilution added to 50 c.mm. of each sample. The infected samples of blood were then transferred to the slide cells and incubated at 37° C. for twenty-four hours. The number of colonies which had grown in each cell were then counted.

Amount of blood in slide cell 50 c.mm.					Number of colonies growing in cell.
Defibrinated blood plus	1/20 of	its	bulk of	0·9 % saline	32
,,	,,	,,	,,	0·25 % citrate	6
,,	,,	,,	,,	0·9 % saline	41
,,	,,	,,	,,	0·25 % citrate	10

Blood was taken as before and defibrinated. The defibrinated blood was allowed to stand on the bench for two hours; the same procedure as before was adopted with a different broth culture of staphylococci.

Amount of blood in slide cell 50 c.mm.					Number of colonies growing in cell.
Defibrinated blood plus	1/20 of	its	bulk of	0·9 % saline	60
,,	,,	,,	,,	0·25 % citrate	19
,,	,,	,,	,,	0·9 % saline	65
,,	,,	,,	,,	0·25 % citrate	32
,,	,,	,,	,,	0·9 % saline	65
,,	,,	,,	,,	0·25 % citrate	30

In all the above experiments the number of colonies growing in the blood to which citrate was added were less than in the blood to which saline was added; therefore citrate has no specific inhibiting effect on the bactericidal power in the concentration used.

The saline was added to the blood in equal bulk to the citrate in order to have exactly comparable dilutions of blood in the slide cells. In these experiments care was taken to add exactly equal quantities of the same dilution of staphylococci in each case. The rate at which the dilution was sucked into the pipette and blown out into the blood, and the rate at which the blood was sucked into the pipette and discharged into the slide cell, was, as far as could be controlled, exactly similar in each case. The rate at which the fluids move along the pipette is of importance, as the number of bacteria adhering to the glass will vary with the rate of movement.

Second Series of Experiments.

A series of experiments was now carried out in which the water content in the cell was the same, but the salt content increasing from 0·5% to 5%.

The procedure adopted was the same as before:

Amount of blood in slide cell 50 c.mm.					Number of colonies growing in cell.
Defibrinated blood plus	1/20 of	its	bulk of	0·5 % saline	60
,,	,,	,,	,,	0·5 % citrate	50
,,	,,	,,	,,	1·0 % saline	40
,,	,,	,,	,,	1·0 % citrate	36
,,	,,	,,	,,	2·0 % saline	37
,,	,,	,,	,,	2·0 % citrate	27
,,	,,	,,	,,	3·0 % saline	54
,,	,,	,,	,,	3·0 % citrate	55
,,	,,	,,	,,	4·0 % saline	46
,,	,,	,,	,,	4·0 % citrate	41
,,	,,	,,	,,	5·0 % saline	60
,,	,,	,,	,,	5·0 % citrate	52

In the second series of experiments, working with increasing salt concentrations and maintaining the same water content, the citrated defibrinated blood has a greater killing power in all cases, except the 3 % salt concentration (which could be accounted for by experimental error). Again, citrate has no specific inhibiting effect on the bactericidal power in the concentrations used.

Third Series of Experiments.

In another series of experiments, blood was taken from a normal subject as before, and divided into two parts. To one part was added 1/20 of its bulk of 5·0 % sodium citrate, which prevented clotting.

The other part was defibrinated and then further divided into three equal parts. To one part was added 1/20 of its bulk of 5·0 % sodium chloride, to another part 5·0 % sodium citrate, and to the third part nothing was added.

A suitable dilution of a twenty-four hours old broth culture of staphylococcus was made, and to 100 c.mm. of each of the four samples of blood 2·5 c.mm. of the same dilution of staphylococcus was added. The 100 c.mm. of each sample was then transferred to the slide cells and incubated as before.

Amount of blood in slide cell 100 c.mm.	Number of colonies growing in cell.
Unclotted blood plus 1/20 of its bulk of 5·0 % citrate	214
Defibrinated blood	64
,, ,, plus 1/20 of its bulk of 5·0 % saline	37
,, ,, ,, ,, 5·0 % citrate	26

In another series, using a greater dilution of staphylococci, the number of colonies growing were :

Amount of blood in slide cell 100 c.mm.	Number of colonies growing in cell.
Unclotted blood plus 1/20 of its bulk of 5·0 % citrate	88
Defibrinated blood	43
,, ,, plus 1/20 of its bulk of 5·0 % saline	20
,, ,, ,, ,, 5·0 % citrate	16

In another series, with a less dilute staphylococcus, the number of colonies growing were :

Amount of blood in slide cell 100 c.mm.	Number of colonies growing in cell.
Unclotted blood plus 1/20 of its bulk of 5·0 % citrate	255
Defibrinated blood	144
,, ,, plus 1/20 of its bulk of 5·0 % citrate	120

In the first and second series of experiments, clotted blood was used in comparing the killing power of citrated serum *plus* corpuscles, with serum *plus* corpuscles, to which sodium chloride was added. In the third series, clotted and unclotted blood was compared with respect to the bactericidal power.

In the first experiment of this series, the number of colonies growing in citrated blood was 214; in clotted blood, 64; in citrated clotted blood, 26.

The clotted blood showed a marked superiority over unclotted blood (citrated), and the clotted citrated blood was superior in killing power to the clotted blood (Fig. 1).

These experiments show that the bactericidal power of blood is greatly

increased by coagulation of the blood. Colebrook and Storer advocated the use of defibrinated blood in "immuno-transfusions," having found that defibrinated blood possessed a marked superiority over citrated blood with respect to the bactericidal power. These observers suggested that the citrate exerted a toxic effect on the leucocytes, and that the toxicity was more marked in unclotted blood than in defibrinated blood. We have also found that defibrinated blood is superior to citrated blood, but in addition we have shown that citrated defibrinated blood is superior to defibrinated blood with respect to the bactericidal power.

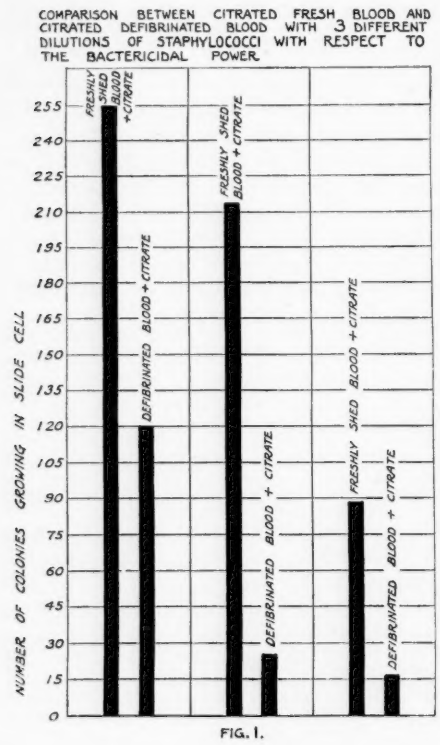

FIG. 1.

It is clear from these results that citrate has no inhibiting effect, and if the leucocytes have a function to perform in the bactericidal action of the blood, the addition of citrate certainly has no toxic influence upon them. We must therefore look for some other cause than anomalous citrate action for the inferiority of citrated unclotted blood.

If we take the experiment again, in which 214 colonies grew in citrated unclotted blood and 26 colonies grew in citrated defibrinated blood, corpuscles were present in both (though in decreased numbers in the case of the defibrinated blood, a certain percentage having been removed by defibrination, as is well known); citrate in equal amounts was present in both.

The difference between the two samples was that in one case the blood had been defibrinated (clotted), and in the other case the blood had not clotted. This greatly increased bactericidal power, then, could only have been conferred on the blood by the process of coagulation.

What bactericidal power, if any, is possessed by circulating blood is another question.

CONCLUSION.

The coagulation of blood enormously increases its bactericidal power Citrate inhibits the growth of staphylococcus in blood.

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ON TETANUS IN THE HORSE SUBSEQUENT TO PROPHYLACTIC IMMUNIZATION; WITH OBSERVATIONS ON RE-VACCINATION.

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THE horse is so very susceptible to tetanus that it is difficult to avoid the occasional occurrence of cases of tetanus amongst the stud of a serum laboratory. Two special measures of precaution may be taken to minimize the risk of such accidents—either (1) the injection at regular intervals of prophylactic doses of tetanus antitoxin, or (2) the active immunization of each animal by means of a series of doses of tetanus toxin guarded by a few doses of antitoxin. The latter method has been employed in these laboratories. It has its obvious advantages, and until recently there has been no cause to doubt that it answered its purpose. The occurrence, however, of tetanus in horses treated in this manner has caused us to modify our opinion, and we feel that details of these cases would be of general as well as special interest.

CASE 1: MARE "*TOISAN.*"

The prophylactic immunization of 4 horses, "*Rose*," "*Toisan*," "*Smilax*" and "*Silene*," was started at the same time, and each received a series of injections as follows:

May 17th, 1921,	1,500	U.S.A. units of tetanus antitoxin intramuscularly.
,, 18th, ,,	15,000	M.L.Ds. (for guinea-pig) of tetanus toxin subcutaneously.
,, 20th, ,,	25,000	M.L.Ds. of tetanus toxin subcutaneously.
,, 23rd, ,,	40,000	,, ,, ,, ,,
,, 26th, ,,	70,000	,, ,, ,, ,,
Total	150,000	,, ,, ,, ,,

This completed the prophylactic treatment against tetanus, and the animals were then used for the production of various sera.

"*Smilax*" was bled out under an anæsthetic on June 26th, 1922, and "*Rose*" on July 20th of the same year. Neither of these animals ever showed any signs of tetanus.

"*Silene*" continued to produce serum until December, 1925, when she died of endocarditis. She had never shown any symptoms of tetanus. Her case forms the subject of a separate communication. All that need be said here is that in June, 1924, her blood did not contain 1/100 unit of tetanus antitoxin per cubic centimetre.

"*Toisan*" received the last dose of an immunization on October 7th, 1924, and was bled on October 14th, 1924.

Note.—Ten cubic centimetres of the serum from this bleeding when injected beneath the skin of a guinea-pig did not produce the slightest symptom of tetanus.

October 21st, 1924. A.M. Showing signs of tetanus. 16,000 U.S.A. units of tetanus antitoxin given intravenously. T. 37·5° C. Pulse 53.

„ 22nd, „ „ About the same. Feeding well. Bowels open. T 37·6°C. Pulse 54.

Noon. Feeding well on boiled oats and bran. Eats all up.

P.M. No change. Having more boiled oats and bran. T. 37·6°C. Pulse 40.

„ 23rd, „ A.M. Still the same; feeding well on same rations. Bowels open. T. 37·6°C. Pulse 45.

Noon. No change.
In afternoon lay down and got up again without assistance.

P.M. T. 37·2°C. Pulse 36.

„ 24th, „ A.M. Seems a bit tired, otherwise no change. T. 37·4°C. Pulse 40.

P.M. No change. T. 37·6°C. Pulse 38.

„ 25th, „ A.M. Still looks tired but does not seem any worse. Feeding same. Bowels open. T. 37·3°C. Pulse 36.

„ 26th, „ 6.15a.m. Found lying down full length. Quite cool. No sweating. Occasional spasms. 5000 U.S.A. units of tetanus antitoxin given intravenously.

11.30a.m. No change either for better or worse.

„ 27th, „ Much the same. Takes oatmeal and water from a bottle. No sweating.

„ 28th, „ Worse. Shot.

In tetanus it seems necessary not to allow any early amelioration of the symptoms to lead to a discontinuance of energetic serum therapy. It is quite possible that this horse might have been saved if more serum had been given on the 22nd, 23rd and 24th, but we thought that once the progress of the disease had been checked by the administration of antitoxin, the animal's own antitoxin-producing mechanism, which already had had some training in the work, would come into play and be sufficient to combat any further toxin production. Unfortunately our hopes were not justified, and we sought for an explanation of this untoward occurrence.

A test of a mixture of bleedings taken between 27 . 7 . 23 and 19 . 2 . 24 showed that at that time she had more than 1/50th but less than 1/10th of a U.S.A. unit of antitoxin per cubic centimetre of her blood. Tests of other samples showed that on 11 . 8 . 24 the antitoxin content was just about 1/100 a.u. per c.c., but on 14 . 10 . 24 the antitoxin content had dropped to less than 1/100 a.u. per c.c. (animal corresponding to 1/100 a.u. died in 36 hours).

It follows that an antitoxin content of this titre is not sufficient to protect a horse against a fatal infection. Then the question naturally arises—What is the quantity of antitoxin which, when present in the blood, will protect a horse against an infection fatal to control animals?

Parker Hitchens (1910) found experimentally that a dose of 300 U.S.A.

units of tetanus antitoxin would protect a horse against an infection fatal to the control animals. Further, Mohler and Eichhorn (1911) showed that as regards a tetanus infection fatal to the controls, 300 U.S.A. units given 48 hours after infection = no symptoms of tetanus, and 700 U.S.A. units given 96 hours after infection = no symptoms of tetanus, while 250 U.S.A. units given 48 hours after infection = local tetanus—recovery.

Now 300 units of antitoxin mixed with the blood volume of an average horse would introduce into the blood approximately 0·01 a.u. per cubic centimetre. But in the experiments quoted above the antitoxin was given subcutaneously, and therefore the antitoxin titre would not reach 0·01 a.u. per c.c. at any time, and so it would appear that less than 0·01 a.u. per c.c. is sufficient to protect.

On the other hand we have the case of "*Toisan*," whose blood at the close of 1923 contained more than 1/50th a.u. per c.c., 10 months later 1/100 a.u. per c.c., and after a further 2 months less than 1/100 a.u. per c.c. It seems to follow that "*Toisan's*" blood must have dropped in titre much more rapidly during the last 2 months than during the previous 10 months, or that an antitoxin titre of about 1/100 a.u. per c.c. is on the border-line of safety. Further, if a difference of 50 units (*i. e.* 0·00015 a.u. per c.c. approximately) in a dose (Mohler and Eichhorn) is the difference between "no symptoms" and "local tetanus," then the margin of safety with these small doses is not great. Another point emerges from a consideration of this case. Mohler and Eichhorn found that 700 a.u. given 96 hours after infection was enough to ward off an attack of tetanus; whereas the 16,000 a.u. given to "*Toisan*" did not prevent a fatal issue. Now 7 days before the onset of symptoms, ten cubic centimetres of "*Toisan's*" blood did not contain enough tetanus toxin to cause any symptoms in a guinea-pig. We may presume, then, that she had not been infected before that date, and that when she received the 16,000 a.u. the infection was not more than 7 days old. Thus we see that while 700 a.u. may be effectual 4 days after infection, more than 20 times that dose was not enough given 3 days later, when symptoms of tetanus were present. This shows most strikingly the absolutely imperative necessity of giving anti-tetanic serum as soon as possible after exposure to the risk of infection.

CASE 2: HORSE "*SYMMERS.*"

The prophylactic immunization of the horses "*Tranquillity*" and "*Symmers*" was carried out in May, 1923. Each had—

May	3rd, 1923,	1,500	U.S.A. units of tetanus antitoxin intramuscularly.
,,	4th, ,,	15,000	M.L.Ds. (for guinea-pig) of tetanus toxin subcutaneously.
,,	7th, ,,	25,000	,, ,, ,, ,,
,,	10th, ,,	40,000	,, ,, ,, ,,
,,	14th, ,,	70,000	,, ,, ,, ,,
,,	17th, ,,	2,000	U.S.A. units of tetanus antitoxin intramuscularly, and 100,000 M.L.Ds. of tetanus toxin subcutaneously.
,,	22nd, ,,	140,000	M.L.Ds. of tetanus toxin subcutaneously.
	Total	330,000	,, ,, ,,

The prophylactic treatment against tetanus was then stopped, and the horses used for the production of other sera. "*Tranquillity*" is alive and well, January, 1926, and has never shown any suspicion of tetanus. The content in tetanus antitoxin of his serum is given in subsequent table.

On March 24th, 1924, "*Symmers*" received a dose of 40 c.c. of a solution of scorpion venom.

April 4th, 1924, Signs suggestive of the onset of tetanus were noticed, and at once 20,000 U.S.A. units of tetanus antitoxin were given intravenously.

„ 5th, „ Definite tetanus, 20,000 a.u. intravenously.

„ 7th, „ Tetanus passing off.

„ 10th, „ The horse seemed quite comfortable, eating well and moving freely. He came to the door of his box, gave two loud snorts and dropped down dead.

Post mortem: Death due to internal hæmorrhage.

In the case of this horse the administration of 390,000 M.L.Ds. (for guinea-pig) of tetanus toxin in six doses did not produce sufficient immunity to protect the animal for one year against infection by the tetanus bacillus. From what has been said above it might be presumed that "*Symmers'*" blood contained less than 0·01 a.u. per c.c., which is in marked contrast to that of "*Tranquillity*." But it seems there is the possibility of another explanation. It is necessary to note that the immunity referred to so far is "antitoxic" immunity.

Now recent work by Tenbroeck and Bauer (1925) has led them to the conclusion that in addition to antitoxin there is in the blood another antibody which is specific for type and which prevents infection. They state that—"Animals that have recovered from a severe generalized tetanus show practically no antitoxin in their serum, but are immune to the type of organism that produced the primary infection. They are not immune to the other types." On this account they question the entire accuracy of Tulloch's (1920) statement that the toxin produced by one type of tetanus bacillus is the same as that produced by any of the other types in that they are all neutralized by one antitoxin. It might then be said that these horses had been infected with a particular strain of tetanus bacillus which had not been used in the preparation of the toxin with which they were treated prophylactically, or of the serum which was administered therapeutically. This would account for the failures, and may have been the case, but against this explanation is the fact that for some years it has been the practice in these laboratories to use in the preparation of tetanus toxin for immunizing purposes more than 8 strains of the tetanus bacillus, including all Tulloch's types.

(*N.B.*—The cultures have been incubated for 1 week at 37°C., and then allowed to remain at room temperature for from 2 to 4 weeks before filtration, as we got the impression that this method of preparation seemed to result in the production of a better antiserum than when the cultures were grown for one week only.)

With the object of throwing more light on this question, some antitetanic serum has been sent out to Dr. Tenbroeck in China in the hope that he may have the opportunity of testing it against the various types of bacilli that

have been isolated there. Meanwhile the blood of several horses has been examined to ascertain the antitoxin content at various periods after prophylactic immunization. The results are gathered together in Table I.

TABLE I.—*Showing the Antitoxin Content of the Blood of Horses at Various Periods after Prophylactic Immunization.*

Name of horse.	Date of prophylactic immunization.	Tetanus toxin.		Date of bleeding.	Period since immunization.	Number of a.u. per c.cm.
		Number of doses.	Total number of M.L.Ds.			
"*Toisan*"	May, 1921	4	150,000	20. 7.23 19. 2.24	30 months	> 1/50 < 1/10.
,,	,,	,,	,,	11. 8.24	38 ,,	> 1/100 < 1/50.
,,	,,	,,	,,	14.10.24	40 ,,	< 1/100.
"*Silene*"	,,	,,	,,	30. 6.24	37 ,,	< 1/100.
"*May*"	February, 1923	6	320,000	14.12.25	34 ,,	Little less than 1/100.
"*Tranquillity*"	May, 1923	,,	390,000	16.12.24	18 ,,	> 1/10 < 1.
,,	,,	,,	,,	9.11.25	30 ,,	More than 10, but less than 20 a.u.
"*Liberal*"	January, 1924	,,	,,	7. 4.25	15 ,,	> 1 < 2.
,,	,,	,,	,,	14.12.25	23 ,,	> 1/50 < 1/10.
"*Election*"	,,	,,	,,	4. 8.25	19 ,,	> 2 < 5.
,,	,,	,,	,,	14.12.25	23 ,,	Over 1; much less than 5 a.u.
"*Conservative*"	,,	,,	,,	7. 4.25	15 ,,	> 1 < 2.
,,	,,	,,	,,	14.12.25	23 ,,	Just 1.
"*Wheatley*"	October, 1924	9	1,010,000	5. 8.25	10 ,,	> 1/10 < 1.
,,	,,	,,	,,	17.12.25	14 ,,	Just under 1/2.
"*Conference*"	,,	8	760,000	5. 8.25	10 ,,	> 1/10 < 1.
,,	,,	,,	,,	17.12.25	14 ,,	Just about 5 a.u.

A consideration of this table makes it obvious that horses vary in their response to prophylactic treatment as much as they do to the more prolonged immunization necessary for the production of therapeutic sera, and that a certain dosage of toxin cannot be relied on to produce a certain antitoxic immunity. An increase in the quantity of toxin administered does not necessarily cause an increase in the amount of antitoxin produced. One important point, however, does emerge, and that is, that the period of antitoxin immunity appears to be limited to about 3½ years, and that at the end of that period the danger-zone is being reached. It follows, then, that to be on the safe side re-vaccination should take place every 3 to 4 years. It was intended to carry this out in the case of "*Silene*" and note the result, but unfortunately she developed a septicæmia, and died of endocarditis and pneumonia.

The question naturally arises as to how one should proceed with the re-vaccination; should it be on the same lines as the original prophylactic treatment, or would fewer doses be enough?

Before the war, while studying the effect of various doses of toxin on horses already hyperimmunized, results were obtained which seem to have some bearing on this question of re-vaccination.

TABLE II.—*Antitoxin Content of the Serum of Horses after each Dose of a Series of Increasing Doses of Diphtheria Toxin; these Animals already possessed a Solid Immunity against the Toxin.*

Number of M.L.Ds. in each dose and intervals between the doses and between samplings.	Number of units of antitoxin per cubic centimetre of serum of horse.									
	"Sidney."	*"Sarah."*	*"Liverpool."*	*"Durham."*	*"Vignette."*	*"Matilda."*	*"Villar."*	*"Fleck."*	*"Kitty."*	*"Tasmania."*
Before re-immunization . . .	< 50	< 50	100 (7 days)	50	< 25	100	50	50	< 25	50
5 days after 18,000 M.L.Ds. . .	50	< 50	150	50	< 25	100	50	50	25	50
5 „ further 36,000 M.L.Ds.	200	100	300	300	100	400	300	200	—	—
5 „ „ 54,000 „ .	—	—	—	—	—	—	—	—	300	200
5 „ „ 72,000 „ .	300	200	500	600	150	500	400	300	—	—
5 „ „ 108,000 „ .	—	—	—	—	—	—	—	—	400	300
5 „ „ 126,000 „ .	600	200	500	—	200	—	—	—	—	—
5 „ „ 189,000 „ .	—	—	—	600	—	500	300	600	300	400
4 „ „ 180,000 „ .	500	—	—	—	150	—	—	—	—	—
4 „ „ 270,000 „ .	—	—	600	—	—	—	—	—	300	300
5 „ „ 180,000 „ .	—	200	—	—	—	—	—	—	—	—
5 „ „ 270,000 „ .	—	—	—	400	—	500	300	500	—	—
7 „ last dose . . .	500	200	600 (10 days)	400	200	400	300	400	300	500
9 „ „ . . .	500	200	500 (15 days)	400	200	400	300	400	200	—
14 „ „ . . .	300	100	500	400	50	400	200	300	200	400
21 „ „ . . .	300	200	500	300	— (23 days)	300	200	300	200	300
25 „ „ . . .	300 (36 days)	100 (27 days)	500	300	100	300	200	300	—	300
28 „ „ . . .	200	100	400	200	100	300	200	300	100	300

First of all it was ascertained that samples of blood taken 5 days after an injection of toxin gave a satisfactory estimate of the effect of the dose upon the production of antitoxin. Then the blood of a series of horses was tested before the commencement of a re-immunization and at 5-day intervals afterwards—a sample being taken before the injection of each dose of toxin. The results are set out in Table II.

It must be borne in mind that these are not first immunizations, but re-immunizations.

It seems that the first dose has usually little effect in raising the antitoxin content of the blood, and, as judged from the results of other experiments not given here, the size of the first dose did not seem to matter, 200,000 or more M.L.Ds. having no more effect than 10,000–20,000 M.L.Ds. The second dose has some effect, but it is the third dose which produces the best result, and it is questionable whether it is worth while administering more than three doses for the purpose of re-vaccination.

The amount of the first dose to be given is a matter for careful consideration and, of course, depends upon the amount of antitoxin in the blood of the animal. For instance, "*May*" at 34 months had less than 1/100 a.u. per c.c. of blood, whereas "*Tranquillity*" at 30 months had 10 units, and there was only a difference of 70,000 M.L.Ds. in the amount of toxin they had received during the prophylactic treatment. Obviously one could start with a larger dose of toxin in the case of the latter.

As "*May*" required re-vaccination it was decided to carry out the previous prophylactic series of injections, and test samples of blood after the first 3 and second 3 inoculations.

"May" Re-vaccination.

Jan. 11th, 1926, 1500 U.S.A. units of tetanus antitoxin intramuscularly.
,, 12th, ,, 150 L^+ doses tetanus toxin subcutaneously.
,, 14th, ,, 250 ,, ,, ,,
,, 16th, ,, 400 ,, ,, ,,
,, 20th, ,, Sampled—antitoxin content of blood = 1/50 a.u.
,, 25th, ,, 1500 a.u. intramuscularly.
,, 26th, ,, 400 L^+ doses tetanus toxin subcutaneously.
,, 28th, ,, 700 ,, ,, ,,
Feb. 1st, ,, 1000 ,, ,, ,,
,, 5th, ,, Sampled—antitoxin content of blood = > 3 < 5 a.u.
,, 9th, ,, 1000 L^+ doses tetanus toxin subcutaneously.
,, 12th ,, 1500 ,, ,, ,,
,, 15th, ,, 2500 ,, ,, ,,
,, 19th, ,, Sampled—antitoxin content of blood = > 10 < 20 a.u.

"Tranquillity" Re-vaccination.

As this horse had a decided amount of antitoxin in his blood the administration of serum was omitted.

Feb. 2nd, 1926, 150 L^+ doses tetanus toxin subcutaneously.
,, 5th, ,, 250 ,, ,, ,,
,, 8th, ,, 400 ,, ,, ,,
,, 12th, ,, Sampled—antitoxin content of blood = > 30 < 50 a.u.

If we take one L^+ dose to be equal to about 100 M.L.Ds. (guinea-pig), we find that after the injection of 80,000 M.L.Ds. "*May's*" serum rose in antitoxin content from just under 1/100 a.u. to 1/50 a.u., and that of "*Tranquillity*" from, we may say, 10 a.u. to 30 a.u. per c.c. It required a further 700,000 M.L.Ds. to bring "*May's*" serum up to 10 a.u. per c.c. This seems a very meagre response to such a large stimulus.

It may be that the interval of time between the vaccination and re-vaccination was too long, and that the body did not respond quickly because it had got "rusty." The idea underlying the use of this last term is that those body-elements which produce the antitoxin behave in much the same manner as the elements which are brought into play for other uses. For instance, when learning to play a game, at first we have to think about the movements we make; our muscles do not respond as we wish, and in comparison with skilled exponents we seem very stupid and awkward, but with practice we become more and more expert until, in time, what we had found difficult is easy and practically automatic. Even when this stage has been reached it requires constant practice to preserve it at the requisite high pitch. Should the opportunity for practice be restricted or removed, then deterioration sets in, the processes cease to be automatic, the skill begins to retrogress, and one is said to become "rusty." The longer the period of "rustiness" continues the more difficult it is to recover the former skill.

Considering the production of antibodies from this point of view, it is possible to account for the different way in which different horses respond to the same course of injections, whether it be a question of re-vaccination or of immunization for the production of therapeutic sera.

SUMMARY.

Experimental and clinical data are supplied which suggest that a course of prophylactic inoculations of tetanus toxin, totalling some 400,000 minimal lethal doses, cannot be relied upon in every case to protect horses against tetanus for more than three years. About this period the antitoxin content of the blood would appear to be on the border-line of safety.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF COLLOIDS.—PART II.

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Experiments detailed in an earlier paper (Steabben, 1925) showed that the injection into normal rabbits of widely differing colloidal substances induced reactions which presented similar features. It was, therefore, reasonable to assume that these changes were of common origin—that is, the effect produced was not, within limits, dependent upon the substance injected, but was rather the expression of a normal physiological activity evoked to deal with an abnormal condition.

Some further experiments on both normal and infected animals are recorded here. Their interest lies, of course, in their relation to non-specific therapy, and for that reason one or two non-colloidal substances used therapeutically are mentioned briefly. It will be recalled that the changes constituting this general reaction were:

(*a*) A rise of temperature, returning to normal within six hours.
(*b*) A temporary increase in the number of erythrocytes per c.mm., followed by a temporary fall below normal.
(*c*) A decrease, both relative and absolute, in the number of polymorphonuclear cells, followed by a general leucocytosis.

SECTION I.—FURTHER OBSERVATIONS ON THE ACTION OF COLLOIDAL AND OTHER SUBSTANCES UPON BLOOD-ELEMENTS AND ANTIBODY CONTENT.

Changes in the Blood-elements and Temperature of Normal Animals.

The results of the present series of experiments are best shown in tabular form (Table I). The same cycle of changes takes place in each case, but the leucopenia varies, as before, with the toxicity of the substance injected. Sodium iodoxybenzoate and manganese chloride have been used in non-specific treatment: in the doses used in these experiments these salts give rise to only a slight direct leucocytosis, with little variation in temperature; they show the characteristic initial rise in the red-cell content, though they are crystalline salts, whereas ferrous sulphate, calcium chloride and sodium citrate—the figures are not given here—cause an immediate fall in the number of red cells per c.mm. In three cases the changes following the injection of *living* bacilli were observed. It has already been shown that a killed bacterial emulsion (typhoid) gives rise to the same sequence of changes as other colloidal suspensions. The usual reaction takes place after the smallest inoculation with *B. pseudotuberculosis*, though a return to normal is

TABLE I.—*Changes in Normal Animals.*

Injection.	Time.	R.B.C. (in mills.).	Leucocytes.	Temperature.	Polymorphs per cent.
5 c.c. gold sol	Before injection	4·93	14,250	102·8	51·9
= 0·23 mgm. Au	15 minutes after	—	11,500	102·5	45·1
	1 hour after	4·86	13,875	103·1	42·5
	2¼ hours after	5·97	15,375	102·8	59·2
	3¼ „	—	23,375	—	62·0
	24 „	5·04	14,875	102·2	39·6
0·5 mgm. $MnCl_2$	Before injection	6·87	6,650	101·7	28·5
	15 minutes after	7·63	4,650	102·3	31·1
	1 hour after	8·08	7,750	102·1	38·7
	2¼ hours after	6·80	6,700	102·2	59·0
	3½ „	6·78	9,750	102·3	66·0
	4¾ „	5·62	8,000	102·2	50 4
	24 „	6·23	9,750	102·3	30·8
Whole agar slope,	Before injection	6·09	6,500	101·2	—
B. pseudo-tuberculosis	15 minutes after	7·25	2,600	101·3	—
	1¼ hours after	6·07	2,500	102·9	—
	2¾ „	7·12	1,600	102·7	—
	4¼ „	6·80	2,500	102·8	—
	24 „	5·50	2,750	102·9	—
10 mgm. *B. tuberculosis*	Before injection	4·72	9,375	103·4	—
	30 minutes after	4·22	4,625	103·8	2·53
	2 hours after	3·99	10,375	104·3	5·66
	3 „	4·36	3,250	105·4	5·50
	4½ „	3·40	1,375	—	0·0
	24 „	4·85	26,375	103·2	30·0
	3 days after	4·30	12,250	103·7	23·2
	4 „	4·15	9,625	103·2	31·2
	7 „	3·09	5,875	105·0	32·0
2 c.c. 8 per cent. white	Before injection	6·64	9,500	102·1	35·0
of egg (= 1 per cent.	15 minutes after	6·50	6,500	101·8	9·0
protein)	2 hours after	—	1,440	103·4	—
	3 „	7·13	1,125	103·5	0·0
	4½ „	5·46	2,375	103·5	15·0
0·05 iodoxybenzoic acid,	Before injection	6·44	7,000	102·0	44·6
neutralized by 1·8 c.c.	15 minutes after	7·41	7,375	99·7	50·3
N/10 NaOH	1 hour after	7·22	11,500	101·2	—
	2½ hours after	6·66	14,625	101·8	80·0
	4 „	5·96	17,500	102·5	89·2
	5⅓ „	6·0	13,750	102·4	—
	24 „	6·28	15,500	101·5	52·0
1/20 agar slope	Before injection	6·02	7,600	104·3	—
B. pseudo-tuberculosis	15 minutes after	5·42	7,000	104·1	—
	1¼ hours after	7·27	8,250	105·0	—
	2½ „	7·13	16,500	—	—
	4 „	4·52	53,600	105·4	—
	24 „	4·46	4,250	106·5	—

out of the question; the larger inoculation in itself evidently produces a severe shock, apart from the progressive destructive power of the organisms. The injection of 10 mgm. of a bovine strain of *B. tuberculosis* caused a severe destruction of both red and white cells, and though there was the ordinary recovery after 24 hours, the blood-counts subsequently made show a progressive anæmia and leucopenia.

Note.—The gold sol used in these experiments was made by reducing a solution containing 0·0046 per cent. of the metal with potassium oxalate; it may produce a very definite reaction, the more noteworthy since the total amount of gold in the 5 c.c. of sol injected is only about one-fifth of a milligramme; this effect must be ascribed to the very extensive and active surface presented by the red gold particles, which do not average more than 10 $\mu\mu$ in diameter.

The other substances are sufficiently described in the table.

Changes in Animals Experimentally Infected.

(a) *Acute infection.*—Six rabbits were inoculated with a large dose (10 mgm.) of a bovine strain of living *B. tuberculosis*, and a few days later the reaction caused by a colloidal injection was determined. It will be seen Table II) in the examples R44 and R46, that after the injection there was

TABLE II.—*Changes in Infected Animals.*

(*a*) CONDITION OF ACUTE INFECTION.

No. of animal.	Injection.	Time.	R.B.C. (in mills.).	Leucocytes.	Temperature.	Polymorphs (per cent.).
R44	5 c.c. 1 per cent. gelatin 5 days after inoculation with 10 mgm. *B. tuberculosis*	Before injection	4·46	6,375	104·6	44·8
		20 minutes after	4·57	12,375	104·6	54·8
		2 hours after	3·70	6,875	105·9	64·1
		3 „	3·48	20,500	103·8	47·4
		4½ „	4·30	21,750	104·2	41·9
		24 „	4·03	8,875	105·0	41·4
		48 „	3·77	7,125	104·7	36·7
R46	5 c.c. 0·5 per cent. agar 6 days after inoculation with 10 mgm. *B. tuberculosis*	Before injection	4·10	10,750	103·8	28·9
		20 minutes after	4·66	13,875	103·8	35·6
		1 hour after	4·20	6,250	105·5	46·3
		3 hours after	3·44	7,625	103·2	36·7
		4½ „	3·40	9,625	102·8	33·0
		24 „	3·77	7,125	104·7	33·4

(*b*) CONDITION OF CHRONIC INFECTION.

R55	5 c.c. gold sol. 19 days after inoculation with 0·04 mgm. *B. tuberculosis*	Before injection	4·47	6,375	102·1	—
		15 minutes after	4·91	5,625	102·1	—
		1½ hours after	4·09	4,625	105·2	—
		3 „	3·62	7,750	104·5	—
		5 „	4·04	4,625	103·8	—
		24 „	3·99	5,310	102·8	—
R59	5 c.c. 1 per cent. casein 5 days after inoculation with 0·04 mgm. *B. tuberculosis*	Before injection	5·72	8,375	102·0	51·5
		15 minutes after	6·02	4,625	102·0	34·5
		1 hour after	6·19	5,000	106·1	42·3
		2¾ hours after	5·52	5,250	104·9	86·0
		4 „	5·30	7,625	105·6	88·4
		5½ „	5·47	6,875	106·2	87·5
		24 „	5·35	10,000	101·8	47·1

not the usual definite rise of at least a million red cells per c.mm., but the number of cells tended rather to decrease still further; the count in each rabbit had already fallen by a million cells per c.mm. from the original count made before inoculation, the figures for which are not given here. The leucocytic changes were anomalous, and did not show the ordered sequence of those in the normal animal. In four cases examined, of which two are given here, there seemed to be a slight immediate leucocytosis and a corresponding increase in the percentage of polymorphonuclear cells, but though the total number of white cells fell again to "normal" or below "normal," the percentage of polymorphonuclear cells appeared to increase. It would seem

here that the lymphocytes and larger mononuclear cells are more actively concerned than normally, since, in the subsequent increase of leucocytes which took place, the polymorphonuclear cells play little part. In these particular cases this specific involvement of the lymphocytes, which is the usual accompaniment to a tuberculous infection, serves to show that, though there appears to be a tentative attempt at a normal response, the ultimate result is to intensify an already existing morbid process (see Section 3). The effect of a colloidal injection in advanced disease might be explained in this way; it suggests, too, why chronic conditions are more amenable to protein therapy. In one instance, however, a marked leucocytosis (9250–33,600) had occurred in 5 hours, though the earlier changes had suggested the same course as the other 3, and the percentage of polymorphonuclear cells had risen from 37·2 per cent. to 77·4 per cent.; this might have been due to the fact that the colloidal injection was given only 3 days after inoculation, when the animal had not deviated so far from the normal. The temperature changes show a range of variation similar to that of normal rabbits, but the actual temperatures are, of course, higher.

(b) *Chronic infection.*—Six rabbits were inoculated with 0·04 mgm. of living bovine tubercle bacilli, and were subsequently given colloidal injections at weekly intervals. The records R55 and R59 show that the reactive power is diminished. The red cell count varies less, but the temperature rises 2–4 degrees, the variations being rather wider than with normal animals. Out of the 3 times in successive weeks that the blood-count R55 was made, a slight leucocytosis occurred twice, but was not present next day; normally, the leucocytosis persists for at least 24 hours. In 4 successive weekly examinations of R59 a leucopenia occurred, the number of white cells returning to the initial level for each count in 24 hours, but a leucocytosis was present only in one instance.

TABLE III.—*Controls.*

Temperatures.

Rabbit No.	104.	105.	106.	103.	104 (*a*).	107.	108.	104 (*a*).	106.	108.
First count*	101·3	102·3	102 6	102·8	103·5	101·7	103·4	103·4	102·3	102·8
15 minutes later	100·3	102·5	102·6	102·9	103·5	102·4	103·4	103·4	102·4	102·8
1 hour later	102·0	103·3	103·0	102·9	—	102·9	—	103·3	102·6	102·9
2½ hours later	100·0	103·6	103·2	103·3	103·5	103·2	103·6	103·4	102·6	103·3
5 „	99·8	—	103·2	103·3	103·5	103·6	103·6	103·3	102·6	103·2

Red cell count in millions.

Rabbit No.	104.	105.	106.	103.	104 (*a*).	107.	108	104 (*a*).	106.	108.
First count	5·03	5·89	6·24	6·73	6·05	6·76	4·90	6·28	6·23	6·07
15 minutes later	5·06	5·95	6·32	7·05	6·09	6·75	—	6·32	6·44	5·74
1 hour later	4·79	6·09	6·48	7·16	5·86	6·52	4·79	6·14	6·61	5·92
2½ hours later	4·37	6·33	5·89	7·25	6·13	6·92	4·68	6·47	6·92	5·53
5 „	4·49	5·48	5·63	6·98	5·71	6·59	4·72	6·15	6·45	6·19

White cell count.

Rabbit No.	104.	105.	106.	103.	104 (*a*).	107.	108.	104 (*a*).	106.	108.
First count	10,625	12,625	10,375	14,750	11,375	8,850	17,375	33,125	10,000	14,250
15 minutes later	10,375	15,125	11,375	14,750	12,625	9,100	—	18,250	12,000	13,500
1 hour later	13,750	13,875	10,500	14,750	13,000	11,000	17,875	21,250	12,125	14,375
2½ hours later	16,250	14,000	10,625	15,750	13,125	12,125	16,500	19,750	17,125	16,000
5 „	13,500	14,625	10,750	16,500	14,125	9,875	18,000	17,375	10,000	12,250

* Rabbit 104 sick, died a week later.

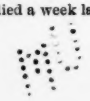


Table III gives the temperature and the red and white cell counts of 10 normal and untreated rabbits, made for the purpose of controlling the experimental determinations. Except for one or two individuals, the results tend to show that, under the conditions of these experiments, the variations in the experimental tables are real and significant changes.

The Effect of Colloids on the Production of Meningococcus Agglutinins in Normal Animals.

In view of the fact that the production of typhoid agglutinins was apparently increased by the injection of a colloidal substance at the same time as the antigen, similar experiments were made with the meningococcus. A Type 1 meningococcus was used, and the emulsion was made with 0·5 per cent. carbol-saline, and killed by heating at 65°C. for 30 minutes. The cultures grown on serum-agar made a more homogeneous emulsion than those grown on trypagar. Table III*a* shows that there was very little difference in the agglutinin production of the treated and control rabbits, although perhaps the average of those receiving colloidal injections was slightly better; (*a*) shows the agglutination titre after 3 serial injections (1000, 2000 and 4000 million organisms) at 4-day intervals, with and without colloid; (*b*) shows the antibody present 6 days after the injection of 2000 million organisms in 6 rabbits with a falling agglutination titre, with and without colloid.

TABLE III*a*.—*Meningococcus Agglutinins.*

(*a*) After 3 serial injections (1000, 2000 and 4000 million organisms) at 4-day intervals with and without colloid.

Colloid	1/200	1/400	1/800	1/1600	1/3200
Gelatin	++	++	++	+	+
Agar	++	++	++	−	−
Nil	+++	+	+	+	−
Silver	+	+	+	−	−
Gold	++	+++	+++	+++	++
Nil	+++	++	+	−	−

(*b*) Rise from falling titre (about 1/400) after 1 injection (2000 million organisms) with and without colloid.

Colloid	1/200	1/400	1/800	1/1600	1/3200
Gelatin	+++	+++	++	+	−
Agar	+++	+++	+++	+	−
Nil	+++	+++	++	−	−
Silver	+++	+++	−	−	−
Gold	+++	+++	+++	++	−
Nil	+++	+++	+++	++	−

SECTION II.—THE ACTION OF COLLOIDS ON PHAGOCYTOSIS (OPSONIN CONTENT), AND ON THE BACTERICIDAL POWER OF WHOLE BLOOD AND SERUM.

A direct leucocytosis would at least suggest that no deleterious change, from the point of view of defence, had taken place in the circulating blood, but a preliminary leucopenia argues a severe disability; either that the white cells are destroyed even more rapidly than they are reinforced, or that the blood is so altered that they cannot function in the peripheral circulation, and so retreat to the lungs, where possibly the conditions are more suitable for their survival. In any case, it is reasonable to suppose that the changes in the peripheral blood which lead to a diminution in numbers of the white cells may be accompanied by changes in the serum which involve a loss of opsonin, though the two factors need not be, and indeed appear experimentally not to be, identical. If opsonin content and leucocyte content are not simultaneously affected in the same direction by the injection of a colloid, then even a marked leucocytosis may have little value for defence; if double the number of leucocytes are present, while the opsonin content is reduced to half, the total

phagocytic effect will not alter, but the energy expended in the production of the extra leucocytes would have to be entered on the debit side of the account.

Phagocytosis and leucocytosis must determine the bactericidal power of the whole blood, but the bactericidal action of the serum has also to be considered. This is probably independent of, and accessory to, the activity of the leucocytic elements of the blood, and it is, in any case, of smaller value. That is to say, a rise in the bactericidal power of the whole blood might occur in conjunction with a fall in the potency of the serum, and yet leave the physiological defences more than normally effective, but it is improbable that a rise in the bacterial power of the serum could compensate a severe leucopenia and loss of phagocytic power.

From the foregoing, it would appear that a direct polymorphonuclear leucocytosis should increase the normal resistance to infection, providing the new cells have a normal or increased phagocytic power,* and it is from this point of view that the following experiments were carried out; they deal with the serum changes which affect phagocytosis, and which result from the injection of colloidal substances. The preparations used were similar to those of other experiments, and included gelatin, casein, gold, agar, iron, sodium nucleate and sodium iodoxybenzoate.

Technique.—Samples of blood were taken from the experimental animals before injection and at definite periods after injection; the control animals were bled at corresponding intervals of time, without other treatment, the blood was allowed to clot, the serum pipetted off and kept in the cold until it was tested for phagocytic power. Human leucocytes were employed.

The emulsion of *Staphylococcus aureus* was made from a 24-hour agar slope culture, and diluted to a standard opacity of about 500 million organisms per c.c.; this gave fairly constant results, of which the following are instances: the pairs of figures show the number of cocci per 50 cells, in experiments made at different times, using two samples of untreated serum from different rabbits with the same staphylococcus emulsion; the last four numbers are the figures for four experiments carried out as a series with the same serum and the same emulsion:

567	554	639	223	442	970	528	545	559	690
513	303	609	339	373	911				

Equal volumes of white cells, emulsion and serum were mixed in sterile tubes and then placed in an electric shaker in the warm incubator for 15 minutes; films were then made and stained by Giemsa's method.

Phagocytosis (Opsonin Content).

The alterations in opsonin content appear in Table IV as ratios, where the number of cocci counted in 100 cells incubated with the first (pre-injection) sample of serum is taken as unity. This standard is arbitrary, but the experiment was, in most cases, controlled. Experiments on both normal and infected animals are included. The figures show that, after the injection of colloidal preparations, the opsonin content of normal animals is increased.

* It is assumed that the new leucocytes during the leucocytosis phase have normally constituted adsorptive surfaces, but it is proposed to deal specially with this matter in later experiments.

TABLE IV.—*Phagocytosis (Opsonin Content).*

(*a*) EFFECT IN NORMAL ANIMALS.

Average time intervals.	Injections. Sod. nucleate.	Casein (R. 47).	Gold.	Casein.	Sod. nucleate.	Gold.	Gelatin.	Gold.	Sod. nucleate.	Casein.	Agar.	Iron sol.
Before injection	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
15 minutes after	2·07	1·00	1·75	1·09	2·56	1·58	1·61	2·79	2·43	1·92	0·14	1·54
1 hour after	1·24	0·61	1·80	1·80	2·34	1·44	1·64	2·87	1·45	1·68	1·99	1·08
2½ hours after	1·18	0·55	2·04	2·11	1·75	1·14	0·87	3·06	1·97	2·95	1·33	0·90
4 „	—	—	1·47	2·20	1·75	1·87	1·29	3·06	0·88	1·33	1·23	0·82
5 „	0·89	1·39	—	—	—	—	1·58	3·32	0·78	1·67	—	—

(*b*) CONTROLS (Injection *nil*).

1st determination	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
15 minutes after	1·75	0·83	0·68	1·24	0·79	0·45	1·28	0·86
1 hour after	1·26	0·73	0·86	1·77	0·41	0·65	1·39	0·77
2½ hours after	1·01	—	0·92	1·68	0·47	0·95	1·19	1·00
4 „	0·71	—	1·44	1·80	0·41	1·41	—	0·70
5 „	—	—	—	—	—	—	—	—

(*c*) ANIMALS INFECTED WITH *B. tuberculosis*.

Average time intervals.	No. of animal and substance injected. R. 60. Agar.	R. 59. Casein.	R. 55. Gold. 3rd week.	4th week.	5th week.
Before injection	1·00	1·00	1·00	1·00	1·00
15 minutes after	0·51	*	1·49	2·08	0·60
1 hour after	1·84	*	2·06	2·00	0·46
2½ hours after	1·02	0·79	1·77	2·04	—
4 „	0·54	0·88	0·80	1·07	0·77
5 „	—	0·88	—	2·00	1·73

* Too many to count.

Except where the more toxic agar and casein were used this rise is immediate, and lasts for some time. In the early stages of infection the animal shows a reactive power similar to that of the normal animal (see R55 and R60); R55, however, reacted differently in a later stage of infection—the fifth week. Whether this was due to the infection this one record cannot show, but this determination of phagocytic power was made two weeks after it was found that the ordinary reactive changes of the blood were lessened (see R55, Table II). In this connection it is to be noted that the only other reaction which does not fall into line is R47 (the second experiment in Table IV). This rabbit was experimentally normal, but a week or two later was unable to move its hindquarters, and a *post-mortem* examination showed multiple growths in both kidneys, numbering about a dozen in each, and varying in diameter from 3 mm. to about 1 cm. The fact that there was no external evidence of illness, taken in conjunction with the results obtained in experimentally infected animals, suggests that the physiological activity measured by these blood changes (following injection of colloids) may index the stage of injury resulting from a morbid process.

TABLE V.—*Bactericidal Power of Whole Blood and Serum.*

(*a*) BACTERICIDAL POWER OF BLOOD AFTER COLLOIDAL INJECTIONS.

Average time intervals.	Injections. Agar.	Agar.	Agar.	Gelatin.	Gold.	Sod. nucleate.	Casein.	Iron.	Sod. nucleate.	Casein.
Before injection	90	Many	200	200 +	230	200	200	450	122	24
15 minutes after	Many	„	Many	80	Many	400	240	600	26	3
1 hour after	50	5	200	70 +	140	100	312	340	74	17
2½ hours after	216	80	1600	80 +	—	140	700	180 +	14	21
4 „	10	20	680	80	300 +	70	—	300	—	9
5 „	—	—	—	80	15	78	350	—	29	—

Controls (No Injections).

Before injection	200	880	88	304	220	62
15 minutes after	500	620	162	300 +	Many	78
1 hour after	500	500 +	82	Many	200	100 +
2½ hours after	500	Many	400	„	—	—
4 „	500	„	—	—	600	Many
5 „	—	—	—	—	—	—

(*b*) BACTERICIDAL POWER OF SERUM AFTER COLLOIDAL INJECTIONS.

Average time intervals.	Injections. Casein.	Peptone.	Sod. nucleate.	Gelatin.	Gold.	Agar.	Gold.	Casein.	Gelatin.	Gold.	Sod. nucleate.	Casein.	Agar.	Iron.
Before injection	199	240	361	76	131	8	16	294	200	94	92 +	19	102 +	Many
15 minutes after	147	463	164	Many	41	Many	16	102	Many	Sterile	79	480	50	„
1 hour after	169	82	388	„	60	4	2	260	„	13	126 +	67	24	600
2½ hours after	157	93	33	„	39	80	—	16	„	200 +	60	340	Sterile	600
4 „	36	80	6	263	53	42	63	Many	„	47 +	106	490	50	600
5 „	—	—	—	—	—	—	—	—	„	Sterile	50	2		—

Controls (No Injections).

Before injection	Many	14	120	114	Many	44	1	167	
15 minutes after	600	70 +	120 +	20	„	133	3	400	
1 hour after	Many	—	400	46	170	76	Sterile	190	
2½ hours after	„	160	242	35	193	160	66	656	
4 „	„	141	320	25	396	544	—	—	
5 „	„	—	—	—	—	—	—	—	

The serum of the control animals bled at corresponding intervals during a period of 5 hours showed little variation in opsonin content.

Bactericidal Power of Whole Blood and of Serum.

In these experiments colloidal preparations of gold, casein, agar, iron, gelatin and sodium nucleate were used, and the injections were made intravenously. To determine the bactericidal power of whole blood, equal volumes of oxalated saline, staphylococcus emulsion and blood taken directly into a capillary pipette from the rabbit's ear were mixed in a small sterile tube and placed in an electric shaker in the incubator at 37°C. for 3 hours; 0·02 c.c. from the sample was then delivered into a Petri dish, mixed with melted agar (54°C.–50°C.) and incubated overnight. The colonies on each plate were then counted, and these are the figures given in the Tables V and VI. The emulsion of staphylococci was a 1–250 *broth* dilution of an overnight broth culture kept at O°C. between the times at which the blood samples were taken.

The serum determinations were made with samples of serum pipetted off from the clot and kept at 0°C. till next day. Equal volumes of staphylococcus broth emulsion and serum were mixed in a sterile tube and incubated at 37°C. for 4 hours; 0·02 c.c. from the sample was then delivered into a Petri dish, mixed with melted agar, and incubated overnight. The colonies on each plate were then counted, and these figures are given in the tables. The emulsion of staphylococci was a 1–21,800 *broth* dilution of an overnight broth culture.

Tables V and VI: Remarks.—There appears to be a rise in the bactericidal power of whole blood and serum, as determined within the limits of these experiments. The fall in the bactericidal power of the blood when casein and agar were used is evidently associated with the leucopenia which these substances cause; in the case of agar, the loss of bactericidal power was apparently greater than with casein, but this may have been the result of the

TABLE VI.—*Phagocytosis and Bactericidal Power.*

GENERAL SUMMARY.

	Injection.								
	Casein.	Sod. nucleate.	Gold.	Gold.	Gelatin.	Sod. nucleate.	Casein.	Agar.	Iron.
Phagocytosis	Rise	Rise	Rise	Rise	Rise	Rise	Rise	Rise	*Nil*
Bactericidal power of—									
(*a*) Serum	Rise	Rise	? Rise	Rise	Fall	Rise	Rise	Rise	Rise
(*b*) Whole blood	—	„	—	„	? Rise	„	Fall	Fall	„

CONTROLS.

Phagocytosis	Rise	Rise	*Nil*	Fall	*Nil*	*Nil*	*Nil*	
Bactericidal power of—								
(*a*) Serum	Fall	Fall	Fall	Fall	Rise	—	Rise	
(*b*) Whole blood	—	„	„	„	Fall	Fall	Fall	

difference in phagocytosis, since Table IV shows that the increase in opsonin after the injection of agar was transient and coincident with the leucopenia, whereas, after the injection of casein, the rise in phagocytic power continued for some time, and was still effective when a leucocytosis was presumably in progress; after the injection of the iron sol, since there was no significant increase, but also no loss in phagocytic power, the leucocytosis must be the factor which raises the bactericidal power of the blood.

The 7 control experiments all show a fall in the bactericidal power of the blood, even though there was once a definite rise in phagocytic power. This may have been due, in some way, to the fact that the animals were bled in quantities of 1·0–1·5 c.c. 5 and 6 times in the day, and it suggests that, for the same reason, the observed effect of colloidal injections upon treated animals may perhaps be less than the real effect.

The figures in Table V(*b*) show the same general tendencies as those of Table V(*a*), but do not yield very much information, except that the bactericidal power of the serum seems to vary independently of that of the whole blood.

Summary of Changes in Phagocytosis and Bactericidal Power.

Table VI shows at a glance the general results obtained from these experiments. It seems justifiable to conclude that colloidal substances may act as stimuli to the normal processes of defence, and at any rate temporarily augment their capacity for effective resistance. It is to be noted that those preparations (gelatin, casein and agar) which cause the more profound and harmful changes in the cellular elements of the blood are also those which are the least effective in their action on phagocytosis and bactericidal power; that is, those which cause a severe leucopenia cause a net loss in the protective value of the blood functions, due solely to the leucopenia, since both the bactericidal power and the opsonic value of the serum are increased in 3 cases out of 4. The results of the control experiments seem to suggest that the repeated withdrawal of even small quantities of blood by venesection may appreciably lower the normal resistance.

SECTION III.—THE INFLUENCE OF COLLOIDAL SUBSTANCES UPON THE COURSE OF EXPERIMENTAL TUBERCULOSIS IN RABBITS.

Chronic conditions are said to benefit most by the methods of colloid therapy, but it is difficult to induce such conditions experimentally. The experiments to be described below were undertaken without knowledge as to whether the treatment would accelerate, delay or terminate the progress of the infection, but the results admit of classification upon the basis of earlier work dealing with the changes occurring in normal animals after the injection of colloidal preparations. The infective agents used were:

(1) An attenuated human strain of *B. tuberculosis* (Lyons), *No. 48, National Collection.*

(2) A bovine strain of *B. tuberculosis* (B. V. 11, Griffiths), *National Collection.*

(3) A strain of *B. pseudotuberculosis rodentium* isolated from a laboratory

rabbit. This organism was found to be too virulent for the purpose in view, but the details of the experiment are given here because they bear upon a different aspect of the subject.

Experiment 1.—Six rabbits were inoculated with 1/500 agar slope of *B. pseudotuberculosis*: 4 were subsequently treated with colloidal injections, 1 and 5 days after inoculation:

No.	Colloid.	Length of survival.	Remarks.
R 14	No colloid	7 days	—
R 27	2 doses colloidal silver	10 days	—
R 38	2 doses agar	5 days	After 2nd dose of agar animal dead in 5 minutes, with convulsions and respiratory distress.
R 34	No colloid	7 days	—
R 32	2 doses gelatin	10 days	—
R 35	1 dose egg white	1 day	Animal dead within 2 hours of injection of colloid. P.M. — Abscesses in liver and lungs; much gas in intestine; lungs showing severe hæmorrhage, with blood-stained fluid in pleural cavity; short Gram-negative bacillus recovered from abscesses in liver and lungs; ? a "natural" infection.

Experiment 2.—Four rabbits were inoculated intravenously with 0·4 mgm. of *B. tuberculosis*, Lyons; colloidal injections were given at the time of inoculation and subsequent injections weekly:

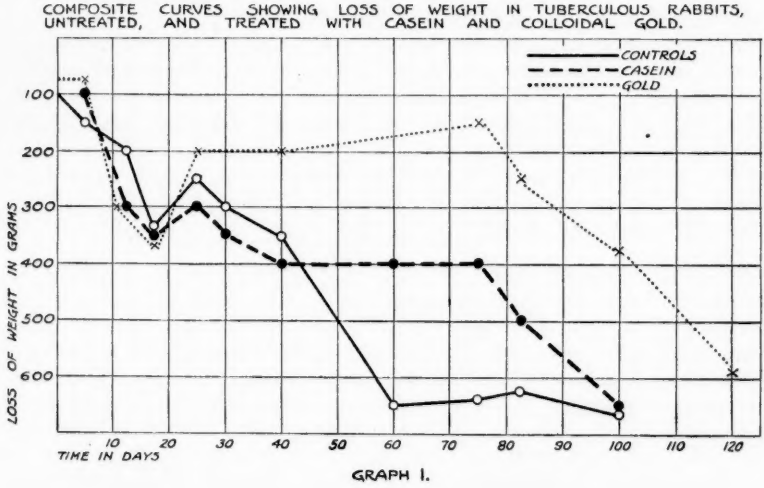
No.	Colloid.	Length of survival.	Remarks.
R 55	Gold	61 days	Weight maintained and rising slightly till 51st day, then sudden fall and death 61st day. P.M. — Degenerate pus in pleural cavity, but no *B. tuberculosis* in pus films. Pericardium thick and white and tough, adherent to wall of thorax. Abscesses in lung-tissue, but none obvious in liver or spleen. No culture in broth or on agar, but a pneumococcus appeared in glucose agar stab.
R 56	*Nil.*	76 days	Steady loss of weight from 15th day. P.M.—Advanced T.B.
R 59	Casein	Killed 76th day	Weight maintained 76 days (1760–1740 gm.) Active and apparently healthy; injections discontinued; sudden loss of weight; animal killed. P.M.—Advanced T.B.
R 60	Agar	34 days	Weight constant 13 days; sudden loss 400 gm. 13th–15th day, death 34th day. P.M. as R 55, but less pus, and lungs hepatized. Similar organism isolated.

Experiment 3.—Twelve rabbits were inoculated intravenously with 0·16 gm. of *B. tuberculosis*, Lyons; colloidal injections were given at the time of inoculation and subsequent injections weekly:

No.	Colloid.	Length of survival.	Remarks.
R 71	Gold	116 days	Severe fall in weight to 14th day; rise to 40th day; stationary to 76th day; fall; died 116th day.
R 79	„	Killed 121st day	Slight fall to 14th day; rising slightly to 108th day; falling 116th day.
R 80	„	Killed 121st day	Slight fall to 14th day; rise to normal and above to 97th day; falling 116th day; lungs not heavily infected.
R 82	„	Killed 123rd day	Weight maintained to 76th day; falling to 116th day.
R 72	Casein	83 days	Steady fall to 40th day; stationary to 60th day; further fall; died 83rd day.
R 76	„	Killed 123rd day	Fall to 40th day; weight recovered and maintained to 108th day; falling to 116th day.
R 78	„	Killed 97th day	Fall to 14th day; weight recovered and maintained to 80th day; falling slightly to 97th day; a few tubercles in lungs, but evidence of infection slight.
R 77	„	Killed 97th day	Fall to 30th day; steady to 80th day; rapid fall.
R 73	Control	Killed 97th day	Steady fall to 60th day; stationary to 97th day.
R 74	„	Killed 97th day	Severe fall to 14th day; rise to 30th day; further fall to 60th day.
R 75	„	Killed 13th day	Sick; no macroscopic lesions.
R 81	„	—	? Uninfected; steady increase in weight to 123rd day (700 gm.).

Experiments 1, 2, 3: *Remarks.*—The dose of *B. pseudotuberculosis* given in Experiment 1 produces an acute infection; whether the injection of gelatin and silver delayed death even for 3 days it is difficult to say, but it is certain that the injections of agar and egg-white accelerated it. The significance of the experiment lies in the violent reactions which occurred (*a*) in a rabbit suffering from an unsuspected natural infection, and (*b*) in one which an experimental infection had had time to develop. The conditions seemed to be those of "fatal colloidal shock," and yet it is not possible that the colloidal injection was its primary cause. In an experiment which is not recorded in detail here, some guinea-pigs were inoculated subcutaneously with 0·1 mgm. of tubercle bacilli, and intramuscular colloidal injections were subsequently given at weekly intervals. In some of these animals after a colloidal injection, there was a marked reaction in the discharging sinuses at the site of inoculation, and the pus cleared up temporarily, the abscess returning to its original condition before the next injection 7 days later. It seems as if the reaction in R38 and R35, Experiment 1, may have been due to some intense focal activity of the same nature, perhaps analogous to "tuberculin shock," as if what is usually a mild reaction becomes an acute fatal shock in a susceptible animal—that is, an animal incapable of reacting normally. What may be termed a "negative" phase occurs immediately after all these injections, in some cases insignificant, but, following substances such as agar and egg albumin, of a severity indexed by the leucopenia. In an animal already subnormal, the severe "shock" (as that of the two cases above) may be a manifestation of the "negative" phase from which the animal has not the capacity for recovery, whereas, normally, recovery is brought about by an exacerbation of the ordinary metabolic processes, which increases their efficiency.

In Experiment 2 the two animals treated with gold and casein kept in better condition than the others for some time. Unfortunately R55 and R60 developed a secondary infection which obscured the findings, but it did seem that the treated animals were able to maintain their weight for some time. Apart from the question of secondary infection, however, agar (R60) seemed less effective, as the lungs of this rabbit were hepatized and showed a more advanced condition of disease than R55. The casein injections in R59 were discontinued after 11 weeks, when the animal's weight fell rapidly and it died in a condition of advanced tuberculosis, but it is not suggested that the sudden final onset of the disease was due to the cessation of injections. If reference is



GRAPH 1.

made to Experiment 3 it will be seen that the same kind of thing occurred. Most of the treated animals maintained weight for a much longer time than the controls, but there seems to come a point where what beneficial effect the colloids may have had is countered by the gradual advance of the infection. It may be here that the colloid becomes harmful rather than useful—if it can be established that it is useful at any stage. Perhaps the resistance of the treated animals is more slowly undermined, but once below a certain degree of reactive power, the colloids tend to accelerate rather than delay the progress of the disease. In Graph 1 the weight curves are composite, and drawn from the averages of each group; they show the general effect of the colloidal injections. No. 81 is not included, as it was not infected.

DISCUSSION.

The experiments in Section 1 are an extension of those recorded in a previous paper (Steabben, 1925); like the earlier ones, they go to show that the effect produced by the injection of colloidal substances is ultimately a function of the reactive power of the organism, and variations in degree of the same

general reaction are dependent on the kind and amount of the substance used. The nature of this reaction involves factors of great delicacy, of whose working little is known, and to say that the injection of colloidal substances accelerates the processes of metabolism, or causes an omnicellular protoplasmic activation, is merely to restate the problem in terms of physiology without contributing to its solution. Recently a good deal of experimental evidence has accumulated, and it may reasonably be hoped that this evidence, whether positive or negative, may ultimately be resolved into accurate knowledge of the contributory factors. At present experimental results are confusing and contradictory, for reasons which will doubtless at some future time be manifest, since there must be some solid basis for certain perplexing facts, both clinical and experimental, which cannot be explained in the light of present knowledge.

Reference was made, in Section 1 of this paper, to sodium iodoxybenzoate and manganese chloride, which have been credited with effects like those of protein therapy. Table I shows that these substances cause the same changes in the blood as colloidal preparations, so that the influence they may have on phagocytosis (Arkin, 1912), antibody production (Arkin, 1915; Wago, 1919; Walbum, 1921; Mackie, 1925; Horgan, 1925) and on infectious processes (Walbum, 1924) might be attributed to an increase of normal activity similar to that following upon a colloidal injection, and not to a specific action of the salt itself.

The experiments of Section 2 show that colloidal injections may increase phagocytosis, and this finding agrees with the work of Kolmer and his colleagues (1917) and Herman (1918). Arloing (1923) refers a temporary "immunity" to *B. pyocyaneus*, following upon a local reaction to the injection of protein, to the leucocytosis alone, but it might have been due, in part, to an increased phagocytic activity of the individual cells. A point which has not previously been referred to here is that, though the onset of leucocytosis is characterized by a preponderance of polymorphonuclear cells, the balance between these and mononuclear cells is usually restored in 24 hours (reference to the white cell table [Steabben, 1925] will make this clear); a leucocytosis is still present, and the increase in the total number of leucocytes, apart from the polymorphonuclear cells, is chiefly made up of large mononuclear cells. These are actively phagocytic, and Permar (1923), by using a method of vital staining, showed that they are derived from the vascular endothelium. The vascular walls are undoubtedly the seat of an intense activity after colloidal injections; the experiments of Petersen (1923) and Harris Boughton (1917) show this. The local reaction at the site of inoculation in a tuberculous guinea-pig is probably one aspect of this vascular disturbance, and the death from "shock" of an animal suffering from an acute infection may also bear some relation to it. If this "shock" followed repeated irritation of the vascular system by protein injections, it might have been referred to the degenerative changes described by Harris Boughton; on the contrary, the symptoms constitute an anaphylactoid syndrome, induced by a single injection of colloid material in the presence of an acute infection. A series of colloid injections seems to accelerate the progress of chronic disease in its later stages, but no one injection of the series causes shock, so that there is some inherent difference underlying the two conditions.

SUMMARY.

1. It is shown that all the substances used cause the same general reaction in normal rabbits, but that, in experimentally infected animals, the characters of the reaction are gradually changed.

2. The injection of colloidal substances into normal rabbits may temporarily increase phagocytosis, and also the bactericidal power of whole blood and serum, but these effects are not constant. Experimentally infected animals lose the power to respond to this stimulation as the infection advances.

3. The injection of a colloidal substance into an animal suffering from an acute infection may cause a fatal reaction, analogous to "shock"; if the animal is in an advanced chronic stage of disease the injection of a colloid may cause a marked focal reaction, and appears to aggravate the condition; in the early stages such an injection seems temporarily to delay the course of infection.

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THE VAN DEN BERGH REACTION IN DIABETES MELLITUS.*

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THE purpose of this communication is to record the observations which were made with reference to the van den Bergh reaction in 130 cases of diabetes mellitus. The results obtained further emphasize the relation which exists between diseases of the biliary passages and diabetes, and also demonstrate the value of this test in the management of the latter disease. The study of this reaction was prompted by the following considerations:

Though the specific nature of diabetes is still unknown, much data is

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available concerning the possible factors which predispose to the development of this disease. One of these, and with which we are now concerned, is disease of the biliary passages. There is much evidence, both clinical and statistical, that the association of diabetes and gall-bladder disease is more than accidental. Pancreatitis is generally recognized as a sequence of cholelithiasis and cholecystitis. The literature on this subject was recently reviewed by Lichty and Woods (1924). Diabetes, as a sequence of acute pancreatitis, is also well recognized. The number of such cases recorded in the literature is increasing. In our clinic there were eight of these, with an average time-interval of 26 months between the acute pancreatitis and the diabetes. An excellent review of this subject was recently recorded by Jones and his co-workers (1925).

Statistical studies demonstrate the relationship between these diseases. The conclusion which has been drawn, by applying the theory of probabilities to concurrent events (Rabinowitch, 1924[1]), was that approximately nine times as many patients with gall-bladder disease had diabetes as would be expected if the two diseases were in no way related. Further suggestive evidence is found in a statistical study by Joslin (1923), who, in a series of 73 cases, found that the average age of his patients at the time of the diagnosis of gall-bladder disease was 45 years, whereas the average age at the onset of diabetes was 47 years. In our series of 22 such cases previously reported (Rabinowitch, 1924[1]), the difference in the average ages at onset for the two diseases was 4·1 years.

From the above observations it appears fairly well established that gall-bladder disease is a definite ætiological factor in diabetes. That it is of sufficient magnitude for serious consideration in preventive treatment is shown by the recent work of Jones *et al.* (1925). In an unselected group of 68 diabetics, cholelithiasis, as diagnosed by examination of duodenal sediments, was found in 19 per cent. of cases. Since the treatment of gall-bladder disease, as compared with diabetes, is relatively more simple, and certainly more satisfactory, and in view of the relationship between both conditions, the necessity of early diagnosis of the former disease is obvious.

The laboratory methods at present available for the detection of disease of the biliary passages are based upon functional alterations from the normal in the gall-bladder, pancreas and liver. Jones *et al.* (1925) record the fact that the rate of disappearance of phenoltetrachlorphthalein from the blood-stream, following its administration intravenously, is not a sufficiently sensitive index of the presence of liver disturbance. Successful X-ray visualization of the gall-bladder with the use of phenoltetraiodophthalein is dependent upon normal liver function. Since gall-bladder disease is very frequently associated with liver disturbance, the limitations of this test are obvious.

Pancreatic disturbances may be detected by methods based upon alterations in the internal and external secretions of this organ. Disturbance of the internal secretion results in hyperglycæmia and glycosuria. Glycosuria is, however, a late phenomenon, and may be influenced by factors other than the degree of hyperglycæmia, particularly altered renal permeability (renal glycosuria, chronic nephritis, etc.). Hyperglycæmia is apparently an early phenomenon. It was found in 62 out of 80 cases (77·5 per cent.) of gall-bladder disease in our series (Rabinowitch, 1924[2]) *during the acute stage* (pain, etc.). Glycosuria was found in one case only. The reason for this is obvious.

The blood-sugar concentrations were below the average threshold value for glucose. They ranged between 0·14 and 0·16 per cent. In the one case of glycosuria the blood-sugar was 0·21 per cent.

Because of this finding, blood-sugar determinations were adopted as a routine in all cases of suspected gall-bladder disease admitted to the hospital. In 56 cases observed subsequently during the acute stages, the percentage incidence of hyperglycæmia was again noted to be high, namely, 66 per cent. In a series of 68 cases observed *in the absence of symptoms* the incidence of hyperglycæmia was only 16·1 per cent. The relative infrequency of hyperglycæmia *in the absence of symptoms* therefore limits the value of this test in the prevention of diabetes.

It is well recognized that even in diabetics, if the disease is mild or under control, no hyperglycæmia may be found in the post-absorptive state. Ingestion of a large quantity (100 gm.) of glucose, however, rarely fails to demonstrate the presence of this disease. For this reason, this procedure was followed in 24 cases of known gall-bladder disease, but in which hyperglycæmia was not found in the post-absorptive state. Of this series, the blood-sugar time curves showed evidence of diminished sugar tolerance in 21 cases. The disadvantages of this test are, however, the necessary preparation (15 hours' starvation) before the test, repeated venous punctures and blood-sugar determinations, which are time-consuming.

Since tests referable to both gall-bladder and pancreatic disturbances lack either sensitivity or are limited in clinical value for other reasons (time-consuming, technical difficulties, etc.), consideration was given to alterations in the liver function. As stated above, the dye test (phenoltetrachlorphthalein) has not been found sufficiently sensitive for the detection of early impairment in liver efficiency. That definite, though slight, impairment in liver function may be detected by other methods was shown by Jones *et al.* (1925): "Bile-pigment elimination in the duodenal contents was found abnormally high in about three-fourths of the cases" of diabetes observed.

Because of the above finding a study was made with reference to the excretion of urobilinogen in the urine. It appeared reasonable to expect that if bile-pigment excretion in the duodenum is increased, the reduction of bilirubin to urobilinogen and reabsorption of the latter would lead to increased urinary excretion. The method employed for the detection of this substance was that described by Wallace and Diamond (1925). This test is sufficiently sensitive, according to Fischer, to show a reaction with a dilution of one part of urobilinogen to 640,000 parts of water. Of 50 cases observed, abnormal urobilinogen excretions were found in only three instances, or 6 per cent. It would therefore appear that in these cases there were either no excesses of pigment excretion into the duodenum, or that the liver or other reticulo-endothelial cells were able to cope with the reabsorbed urobilinogen, re-converting it into bilirubin.

Since the van den Bergh reaction (van den Bergh and Snapper, 1913) was brought to our attention by McNee (1922, 1925) it has received wide application. Observations made in this hospital have shown that individuals may have bilirubin in the blood-stream in amounts greater than the normal, and still show no increased excretion of urobilinogen in the urine. This

probably explains the discrepancy between our observations on urobilinogen excretion in the urine and those of Jones *et al.* on bilirubin excretion in the duodenum. Since in the absence of hæmolytic processes or biliary obstruction, excess bilirubinæmia may be regarded as being indicative of defective function of liver-cells, and in view of the finding of alteration in hepatic activity in diabetes by Jones, the van den Bergh reaction was observed in a series of 130 diabetics. The *indirect* method (in alcoholic solution) was employed.

The technique has been described frequently and need not be repeated here. It might, however, be observed that because of the sensitivity of this reaction, normal individuals may show faint but definite positive reactions. A distinct, though slight, reaction was found in 23 of 33 medical students. The indirect method, however, allows quantitative determinations. The actual number of units was not determined in our cases. Since the maximum concentration found in normal individuals is known, the intensity of the pink colour due to this concentration was accepted as the standard. Colours of greater intensity were regarded as indicative of excess bilirubinæmia, and these are referred to in this discussion as positive reactions.

A controlled group of subjects was first observed. This consisted of 221 patients attending the Medical Out-Patient Clinics. Since all patients known to have diabetes are referred by the admitting officer to the Diabetic Clinic, it was assumed that the great majority of these subjects had no diabetes. None of these individuals could be regarded as perfectly normal. A positive van den Bergh reaction was obtained in 46 cases. The diagnoses of these are recorded in Table I.

TABLE I.

Diagnosis.	Number of cases.
Catarrhal jaundice	2
Gall-bladder disease	9
Heart failure	13
Carcinoma	1
Cirrhosis of liver	2
Peptic ulcer (?) ; suspected gall-bladders	6
Acute infections:	
Tonsillitis	1
Pulmonary T.B.	4
Pleurisy	1
Chronic appendicitis	2
Constipation	1
Pregnancy	1
Lues	1
Menopause	2

It will be noted that in the great majority of instances a positive reaction may reasonably have been expected. This gave further proof of the value of this test. Its value in detecting disease of the gall-bladder and its passages may readily be seen from Table II, in which are recorded the van den Bergh reactions and blood-sugars, determined simultaneously, in 124 patients with this disease.

TABLE II.—*Incidence of Hyperglycæmia and Hyperbilirubinæmia in Biliary Disease.*

	Number of cases.	Positive van den Bergh.		Hyperglycæmia.	
		Number.	Per cent. incidence.	Number.	Per cent. incidence.
Cholelithiasis and cholecystitis (with symptoms)	56	48	85·7	37	66·0
(no symptoms)	68	31	45·6	11	16·1
Pancreatitis	22	20	90·9	18	81·6

It will be noted that *in the presence of symptoms* of cholelithiasis and cholecystitis, both the van den Bergh reactions and blood-sugar tests were positive in a large number of cases. The former test was, however, more sensitive. The percentage incidences of positive reactions were 85·7 and 66·0 respectively. *In the absence of symptoms* the van den Bergh reaction indicated disease in practically three times as many cases as the blood-sugar determinations. The remarkable incidence of positive reactions in the cases of pancreatitis further demonstrates the influence of the pancreas upon hepatic activity.

In view of the above findings only those diabetics were selected who had no obvious disease to account for hyperbilirubinæmia (heart failure, pernicious anæmia, etc.). Of the group of 130 cases, a positive (indirect) van den Bergh reaction was found in 34 individuals, or 26·1 per cent. This agrees rather closely with the incidence of cholelithiasis found by Jones in his group of diabetics, which was 19 per cent. It is, however, much below that found by that author for bile-pigment elimination in the duodenum.

As the subjects were divided according to their body-weights into those over weight and those of normal or under weight for their heights, an interesting result was obtained. This is recorded in Table III.

TABLE III.—*Body-weight : Bilirubinæmia Relationship.*

	Number of cases.	Positive van den Bergh.	
		Number.	Per cent. incidence.
Over weight	87	28	32·1
Normal or under weight	43	6	13·9

It will be noted that the percentage incidence of positive reactions was found to be over twice as high in the over-weight subjects as in those normal or under-weight. The relationship is very suggestive: over-weight individuals are susceptible to gall-bladder disease; over-weight individuals are susceptible to diabetes.

SUMMARY.

The van den Bergh reaction, known to be a sensitive test for the detection of excess bilirubinæmia, was applied to a group of individuals with biliary disease.

The incidence of positive reactions found suggested that the liver escapes injury in a minority of such cases only.

Because of this finding and the known relationship between disease of the biliary passages and diabetes, the reaction was observed in a series of diabetics.

Of a group of 130 diabetics, positive van den Bergh reactions were found in 34 cases—an incidence of 26 per cent. This incidence agrees closely with that found in cholelithiasis in the 68 cases of diabetes observed by Jones, Castle, Mulholland and Bailey.

Since gall-bladder disease is proven to be an important ætiological factor in the production of diabetes, and in view of the fact that as compared with diabetes, it can be more simply, and certainly more satisfactorily, treated, the necessity of a simple test for its detection is obvious. The van den Bergh reaction is of value for this purpose. The routine performance of this test may, therefore, be of assistance in the prevention of diabetes.

As with all other laboratory tests, it is only of value when properly interpreted. For the latter purpose, correlation with the clinical picture is essential in order to exclude conditions other than gall-bladder disease and pancreatitis (hæmolytic processes, heart failure, etc.) which may cause positive reactions.

Grateful acknowledgment is due to the chiefs of the various services in the hospital for their co-operation. It was by means of this that blood analyses were made possible in practically all patients with biliary disease admitted to the wards, medical and surgical. Thanks are also due to the Chiefs of the various medical out-patient clinics for the material made available for the "control" group of cases.

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